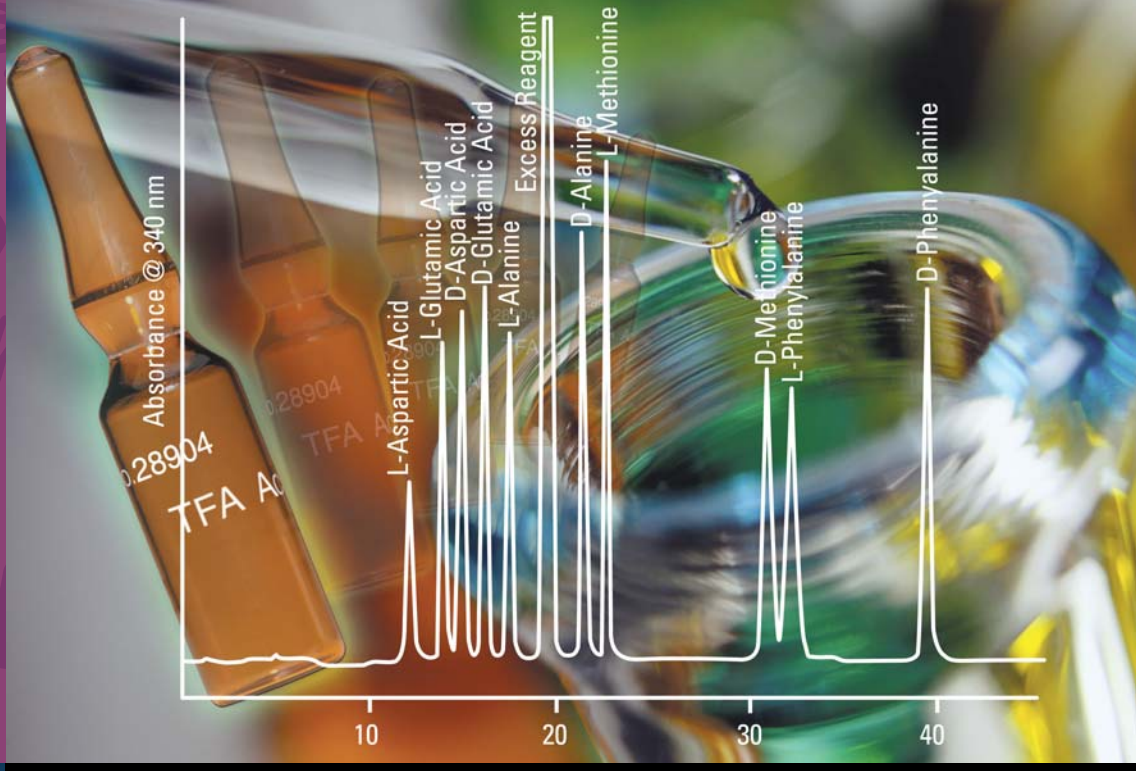


# Thermo Scientific Pierce GC and HPLC Technical Handbook

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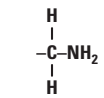
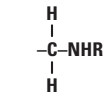
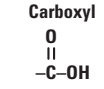
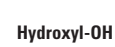
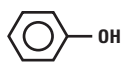
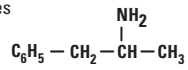
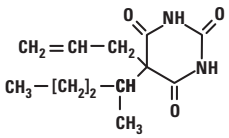
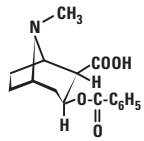
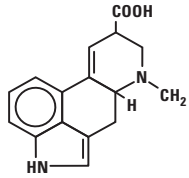
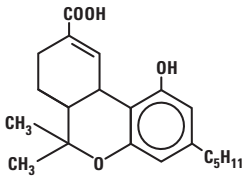
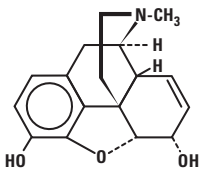
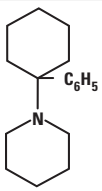
# Table of Contents

# Derivatization Reagents for Specific Functional Groups

| Functional Group | Procedure | Reagent | Derivative | Notes |
|---|---|---|---|---|
| **Amides**  Primary  Secondary | Silylation | BSA | TMS Amides | Difficult to form due to steric hindrance |
| | | BSTFA | TMS Amides | |
| | | BSTFA+TMCS | TMS Amides | TMCS used as a catalyst |
| | | MSTFA | TMS Amides | Reaction byproducts more volatile |
| | | MSTFA+TMCS | TMS Amides | |
| | | Tri-Sil Reagents | TMS Amides | |
| | | MTBSTFA | TBDMCS Amides | Difficult to form; very stable |
| | | MTBSTFA+TBDMCS | TBDMCS Amides | TBDMCS aids derivatization |
| | Acylation | MBTFA | Trifluoroacetamides | |
| | | TFAA | Trifluoroacetamides | |
| | | PFAA | Pentafluoropropionamides | Good for ECD detection |
| | | HFBA | Heptafluorobutyamides | |
| | Alkylation | MethElute Reagent (TMPAH) | Methyl Amides | On-column derivatization especially for drugs |
| **Amines**  Primary  Secondary | Silylation | BSA | TMS | |
| | | BSTFA | TMS | |
| | | BSTFA+TMCS | TMS | TMCS aids derivatization |
| | | MSTFA | TMS | |
| | | MSTFA+TMCS | TMS | TMCS aids derivatization |
| | | Tri-Sil® Reagents | TMS | |
| | Silylation | MTBSTFA | TBDMCS | Difficult to form, but more stable |
| | | MTBSTFA+TBDMCS | TBDMCS | TBDMCS aids derivatization |
| | Acylation | MBTFA | Trifluoroacetamides | Good for trace analysis with ECD |
| | | TFAA | Trifluoroacetamides | Good for trace analysis with ECD |
| | | TFAI | Trifluoroacetamides | Good for trace analysis with ECD |
| | | PFAA | Pentafluoropropionamides | |
| | | PFPI | Pentafluoropropionamides | |
| | | HFAA | Heptafluorobutyamides | |
| | | HFBI | Heptafluorobutyamides | |
| | Alkylation | MethElute Reagent (TMPAH) | Methyl Amides | On-column derivatization for specific drugs |
| **Carbohydrates**  | Silylation | MSTFA | TMS | |
| | | TMSI | TMS | Can be used with some syrups |
| | | Tri-Sil Reagents | TMS | |
| | Acylation | MBTFA | Trifluoroacetates | Volatile derivatives of mono-, di- and trisaccharides |
| | | TFAI | Trifluoroacetates | |
| **Carboxyl**  | Silylation | BSA | TMS | Easily formed, generally not stable, analyze quickly |
| | | BSTFA | TMS | |
| | | BSTFA+TMCS | TMS | |
| | | MSTFA | TMS | |
| | | TMCS | TMS | Can be used with some salts |
| | | TMSI | TMS | |
| | | Tri-Sil Reagents | TMS | |
| | | MTBSTFA | TBDMCS | More stable than TMS derivatives |
| | | MTBSTFA+TBDMCS | TBDMCS | TBDMCS aids derivatization |
| | Alkylation | PFBBr | Pentafluorobenzyl Esters | Used in EC detection & UV, MS |
| | | $BF_3$-Methanol | Methyl Esters | Best for large samples of fatty acids |
| | | Methylate Reagent (DMFDMA) | Methyl Esters | Fatty acids and amino acids |
| | | MethElute Reagent (TMPAH) | Methyl Esters | On-column derivatization |
| | | PFAA+Pentafluoropropanol | Pentafluoropropyl Ester | Drug analysis |
| **Hydroxyl-OH**  Alcohols  Phenols | Silylation | BSA | TMS | Most often used derivatives |
| | | BSTFA | TMS | Good thermal stability |
| | | BSTFA+TMCS | TMS | Poor hydrolytic stability |
| | | HMDS | TMS | Weak donor usually used with TMCS |
| | | MSTFA | TMS | |
| | | MSTFA+TMCS | TMS | |
| | | TMCS | TMS | Weak donor usually used with HMDS; can be used with salts |
| | | TMSI | TMS | Can be used with syrups |
| | | Tri-Sil Reagents | TMS | |
| | | MTBSTFA | TBDMCS | More stable than TMS, good MS fragmentation patterns |
| | | MTBSTFA+TBDMCS | TBDMCS | TBDMCS aids derivatization |
| | Acylation | MBTFA | Trifluoroacetates | Good for trace analysis with EDC |
| | | TFAA | Trifluoroacetates | Good for trace analysis with EDC |
| | | TFAI | Trifluoroacetates | Good for trace analysis with EDC |
| | | PFAA | Pentafluoropropionates | Good for trace analysis with EDC |
| | | HFBI | Heptafluorobutrates | Good for trace analysis with EDC |
| | | HFAA | Heptafluorobutrates | Good for trace analysis with EDC |
| | Alkylation | PFBBr | Pentafluorobenzyl Ethers | With alkoxides only |

# Derivatization Reagents for Drugs-of-Abuse

| Drug | Form | Reagent | References |
|---|---|---|---|
| Amphetamines  | Amphetamines | BSTFA | 1 |
| | Amphetamines | HFAA | 2-5 |
| | Amphetamines | HFAA/PFAA | 6 |
| | Amphetamines | MSTFA with TMCS | 7 |
| | Amphetamines | TFAA | 7,8 |
| | Methamphetamine | TFAA | 9,10 |
| Barbiturates  | | BSTFA | 1 |
| | | MethElute Reagent (TMPAH) | 11-13 |
| | | Methylate Reagent (DMFDMA) | 14,15 |
| | | PFBBr | 16 |
| Cocaine  | Benzoylecgonine | BSTFA/Butyl Iodine/TMAH | 17 |
| | | BSTFA | 1,18 |
| | | MTBSTFA | 19 |
| | | PFAA/PFPOH | 9,20 |
| LSD  | | BSA | 21 |
| | | BSTFA | 22 |
| | | MSTFA | 21 |
| | | TFAI | 23 |
| Marijuana  | THC metabolites | BSA | 24 |
| | | BSTFA/BSTFA+1% TMCS | 24-27 |
| | | BSTFA/TMCS/TMSI | 24 |
| | | MSTFA | 9 |
| | | MSTFA/MSTFA+1% TMCS | 27 |
| | | MTBSTFA | 28 |
| | | PFBBr | 29 |
| | | PFAA/HFIOH | 30 |
| | | PFAA/PFPOH | 31 |
| | | TFAA & $BF_3$/MeOH | 32 |
| | | MethElute Reagent (TMPAH) | 9 |
| | | TMSI | 24 |
| Opiates  | Morphine | BSTFA+1% TMCS | 33 |
| | | MBTFA | 34 |
| | | PFAA | 35 |
| | | TFAA | 36 |
| | Morphine/Codeine | BSTFA | 1,37 |
| | | BSTFA+1% TMCS | 38,39 |
| | | BSTFA/TFAI | 40 |
| | | HFBA | 38 |
| | | MBTFA | 38 |
| | | PFAA | 38,41 |
| | | PFAA/HFAA | 37 |
| | | PFAA/PFPOH | 9 |
| | | TFAA | 42 |
| | | Trimethylsilyl | 43 |
| PCP  | PPC/PCHP/PCP | BSTFA+1% TMCS | 44 |
| | | HFAA | 45 |

*See references on following page.*

*† Reagent names correspond to product names as listed in this catalog, except PFPOH (pentafluoropropanol).*

*HFIOH (heptafluoro-isopropanol) is not offered by Thermo Fisher Scientific. PFAA (PentaFluoropropionic Acid Anhydride) and HFAA (HeptaFluorobutyric Acid Anhydride) are sometimes incorrectly referred to as PFAA and HFBA (respectively), which are the appropriate abbreviations for the free acid.*

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## Drugs-of-Abuse Derivatization Applications

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Crouch, D.J., *et al.* (1995). *J. Anal. Toxicol.* **19**, 352-358.
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**Derivatization of Cannabinoids**

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# Introduction to **Gas Chromatography**

## Derivatization

**The chemical literature contains an abundance of data on derivatization, most of which is relevant to particular compounds, classes of compounds and derivatization reagents. Two books are recognized as standards in the field of analytical derivatization. The first book, *Handbook of Analytical Derivatization Reactions* by Daniel R. Knapp[1], provides a general collection of analytical derivatization methods for chromatography and mass spectroscopy (MS) that involves formation of covalent derivatives prior to analysis. The second book, *Silylation of Organic Compounds* by Alan F. Pierce,[2]" was a significant factor in the transfer of silylation reactions from the relatively esoteric field of organosilicon chemistry to the status of perhaps the most widely practiced of derivatization methods."[3]**

Compounds or compound mixtures are derivatized before analysis for the following reasons:

1. To make a compound that otherwise could not be analyzed by a particular method suitable for analysis[4]
2. To improve the analytical efficiency of the compound[5,6]
3. To improve the detectability of the compound[7]

## Suitability

Often compounds cannot be analyzed because they are not in a form that is suitable for the particular analytical technique. Examples include nonvolatile compounds for GC analysis,[8,9,10] insoluble compounds for HPLC analysis and materials that are not stable using the conditions of the technique.[11] The derivatization procedure modifies the chemical structure of the compounds, allowing analysis by a desired technique.[12]

## Efficiency

Direct analysis can be difficult when compounds interact with each other or with the column. These interactions can lead to poor peak resolution and/or asymmetrical peaks that make proper peak integration difficult or impractical. This interference can be reduced with conversion to derivatized products.[13,14] Compounds that exhibit co-elution can often be separated by using the appropriate derivatization methods.

## Detectability

As demand increases for the analysis of increasingly smaller amounts of materials, it becomes important to extend the detectability range of the materials in question. This increased sensitivity can be accomplished by improved detector design that is directed toward specific atoms or functional groups.

Another popular approach to increase detectability is the use of derivatization. Enhanced detectability can be achieved by increasing the bulk of the compound, or by introducing atoms or functional groups that strongly interact with the detector.[16,17] This technique is performed in gas chromatographic applications, with the addition of halogen atoms for electron capture detectors,[18,19] and with the formation of TMS derivatives to produce readily identifiable fragmentation patterns and mass ions.[20]

## Types of Derivatization

Compounds containing functional groups with active hydrogens (-COOH, -OH, -NH and -SH) are usually derivatized for analysis by gas chromatography. These functional groups have a tendency to form intermolecular hydrogen bonds that affect the volatility, their tendency to interact deleteriously with column packing materials and their thermal stability. Silylation, acylation and alkylation are derivatization techniques used to alter these functional groups to improve their thermal and chromatographic character.

The ideal derivatization procedure will:

1. Accomplish the desired modification.
2. Proceed quantitatively, or at least reproducibly.
3. Produce products that are readily distinguishable and separable from the starting materials.
4. Proceed rapidly with simple and straight-forward laboratory techniques that will be both selective and applicable to a number of similar compounds.
5. Involve reagents and reactions that present no unusual hazards.

## Silylation and Silylation Reagents

*Only Thermo Scientific Pierce Reagents offer the combination of variety, quality and reliability.*

Silyl derivatives are the most widely used derivatives for gas chromatographic applications. Usually they are formed by the replacement of the active hydrogens from acids, alcohols, thiols, amines, amides and enolizable ketones and aldehydes with the trimethylsilyl group. A variety of reagents is available for the introduction of the trimethylsilyl group. These reagents differ in their reactivity, selectivity and side reactions and the character of the reaction products from the silylation reagent itself. Considerable literature is available to assist you in the selection of the most suitable silylation reagent for your particular compounds or systems.[1,2]

Silylation reagents and trimethylsilyl derivatives are hydrolytically unstable and must be protected from moisture. However, the rate of hydrolysis for various reagents and derivatives is different, and sometimes it is possible to prepare derivatives in the presence of small amounts of moisture,[21] or to isolate and purify derivatives by extraction in an organic solvent, followed by washing with aqueous solutions.[22] Reagents that introduce a *t*-butyldimethylsilyl group instead of the trimethylsilyl group were developed for greater hydrolytic stability.[23] These derivatives provide improved stability against hydrolysis and provide distinctive fragmentation patterns, making them useful in GC/MS applications.[24]

Most trimethylsilyl and *t*-butyldimethylsilyl derivatives offer excellent thermal stability and are suitable for a wide range of injector and column conditions. However, as the silylation reagents will derivatize nearly all active hydrogens, it is important that they are not injected onto any column in which the stationary phase contains these functional groups. Examples of packings that are not compatible with silylating reagents are polyethylene glycols (Carbowax® Glycols and Superwax) and free fatty acid phases (FFAP).

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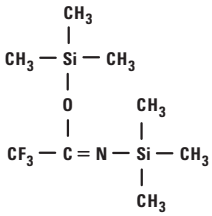
## BSTFA

*For excellent chromatographic separations.*



**BSTFA**
MW 257.4
bp 40°C/12 mm
$d_4^{20}$ 0.961

The greatest advantage of using Thermo Scientific Pierce BSTFA over other silylating reagents is the increased volatility of its byproducts, mono(trimethylsilyl) trifluoroacetamide and trifluoroacetamide. This increased volatility results in the byproducts eluting with the solvent front, providing excellent chromatographic separations.

BSTFA is a powerful trimethylsilyl donor, with donor strength that is comparable to its unfluorinated analog BSA [*N,O*-Bis(trimethylsilyl)acetamide]. BSTFA reacts to replace labile hydrogens on a wide range of polar compounds with a -Si$(CH_3)_3$ group. This physical characteristic is particularly useful in the gas chromatography of some lower boiling TMS-amino acids and TMS Krebs cycle acids.

### PROTOCOL

1. Combine 5-10 mg sample, 0.5 ml Pierce BSTFA and 1.0 ml solvent (acetonitrile is recommended for amino acids) in a 3.0 ml Thermo Scientific Reacti-Vial™ Small Reaction Vial.
2. Cap vial and shake for 30 seconds.
3. Heat at 70°C for 15 minutes.
4. Analyze by gas chromatography.

**NOTE:** This protocol is not recommended for sugars.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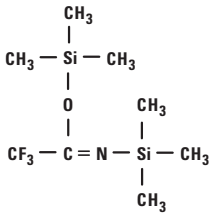
## Ordering Information

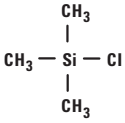
| Product # | Description | Pkg. Size |
|---|---|---|
| **38828** | BSTFA [*N, O*-bis (trimethylsilyl)trifluoroacetamide] | 25 g Hypo-Vial Sample Storage Vial |
| **38829** | BSTFA | 100 g Hypo-Vial™ Sample Storage Vial |
| **38830** | BSTFA | 10 x 1 ml ampules |

## BSTFA + TMCS

*The reagent to choose for difficult-to-silylate compounds.*



**BSTFA**
MW 257.4
bp 40°C/12 mm
$d_4^{20}$ 0.961



**TMCS**
MW 108.7
bp 57.6°C
$d_4^{20}$ 0.858

Thermo Scientific Pierce BSTFA + 1% TMCS is ideal for derivatizing fatty acid amides, slightly hindered hydroxyls and other difficult-to-silylate compounds. This catalyzed formulation is stronger than BSTFA alone.

### PROTOCOL

1. Combine 5-10 mg sample, 0.5 ml Pierce BSTFA + 1% TMCS and 1.0 ml solvent (acetonitrile is recommended for amino acids) in a 3.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Cap vial and shake for 30 seconds.
3. Heat at 7°C for 15 minutes.
4. Analyze by gas chromatography.


**References**

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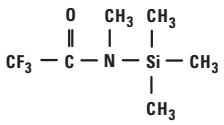
## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **38831** | **BSTFA + 1% TMCS [*N-O*-bis (trimethylsilyl)trifluoroacetamide + 1% Trimethylchlorosilane]** | 10 x 1 ml ampules |
| **38832** | **BSTFA + 1% TMCS** | 10 g Hypo-Vial Sample Storage Vial |
| **38833** | **BSTFA + 1% TMCS** | 25 g Hypo-Vial Sample Storage Vial |
| **38834** | **BSTFA + 1% TMCS** | 100 g Hypo-Vial Sample Storage Vial |
| **38840** | **BSTFA + 10% TMCS [*N-O*-bis (trimethylsilyl)trifluoroacetamide + 10% Trimethylchlorosilane]** | 10 x 1 ml ampules |

## MSTFA[1-9] and MSTFA 1% TMCS[10-12]

*Offers maximum volatility.*



**MSTFA**
MW 199.1
bp 70°C/75 mm
$d_4^{20}$ 1.11

### Highlights:

- Trimethylsilyl donor strength comparable to BSA and BSTFA
- Reacts to replace labile hydrogens on a wide range of polar compounds with a -Si$(CH_3)_3$ group
- Used to prepare volatile and thermally stable derivatives for GC and MS
- Primary advantage of Thermo Scientific Pierce MSTFA is the volatility of its byproduct, N-methyltrifluoroacetamide; MSTFA is the most volatile TMS-amide available which has an even lower retention time than MSTFA
- Often TMS derivatives of small molecules can be analyzed when derivatized with MSTFA because the byproducts and reagent itself usually elute with the solvent front
- Addition of Thermo Scientific Pierce TMCS aids derivatization of amides, secondary amines and hindered hydroxyls not derivatized by MSTFA alone

MSTFA is the most volatile TMS-amide available - its even more volatile than BSTFA or BSA.[1] Its byproduct, N-methyltrifluoroacetamide, has a lower retention time in GC applications than MSTFA itself. This makes it ideal for GC determinations in which the reagent or byproducts may obscure the derivative on the chromatogram. Silylation of steroids shows MSTFA to be significantly stronger in donor strength than BSTFA or BSA.[2] MSTFA will silylate hydrochloride salts of amines directly.

### PROTOCOL

1. Combine 5-10 mg sample, 0.5 ml Pierce MSTFA and 1.0 ml solvent (acetonitrile is recommended for amino acids) in a 3.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Cap vial and shake for 30 seconds.
3. Heat at 70°C for 15 minutes.
4. Analyze by gas chromatography.

**References**

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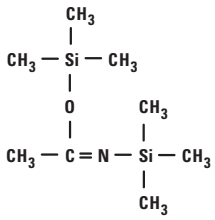
## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 48910 | MSTFA (*N*-Methyl-*N*-trimethylsilyltrifluoroacetamide) | 10 x 1 ml ampules |
| ✖ 48911 | MSTFA | 10 g Hypo-Vial Sample Storage Vial |
| ✖ 48913 | MSTFA | 25 ml Hypo-Vial Sample Storage Vial |
| ✖ 48914 | MSTFA | 100 ml Hypo-Vial Sample Storage Vial |
| ✖ 48915 | MSTFA + 1% TMCS (*N*-Methyl-*N*-trimethylsilyltrifluoroacetamide+ 1% Trimethylchlorosilane) | 10 x 1 ml ampules |

✖*Additional hazardous handling charge.*

## BSA

*The perfect reagent for volatile TMS derivatives.*



**BSA**
MW 203.4
bp 71-73°C/35 mm
$d_4^{20}$ 0.832

Under relatively mild conditions, Thermo Scientific Pierce BSA reacts quantitatively with a wide variety of compounds to form volatile, stable TMS derivatives for GC analysis. BSA is used extensively for derivatizing alcohols, amines, carboxylic acids, phenols, steroids, biogenic amines and alkaloids. It is not recommended for use with carbohydrates or very low molecular weight compounds.

BSA is used in conjunction with a solvent such as pyridine or DMF, and reactions are generally rapid. When used with DMF, BSA is the most suitable reagent for derivatizing phenols. A study of the silylating properties of BSA made by Klebe, Finkbeiner and White[1] showed the following reactions with BSA:

- Amino acids to form both *N,O*-bonded TMS derivatives
- Hydroxyl compounds to form TMS ethers
- Organic acids to form TMS esters
- Aromatic amides to form N-TMS derivatives

### PROTOCOL 1

1. Combine 5-10 mg sample, 0.5 ml Pierce BSA and 1.0 ml solvent (acetonitrile is recommended for amino acids) in a 3.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Cap vial and shake for 30 seconds.
3. Heat at 70°C for 15 minutes.
4. Analyze by gas chromatography.

### PROTOCOL 2

This method was developed by E.M. Chambaz and E.C. Horning for the silylation of hydroxyl groups in sterically unhindered positions in steroids. This includes sites such as 3, 7, 16, 17(sec), 20 and 21 positions in the steroid structure. This method may be used for silylating many hydroxyl and polyhydroxyl compounds other than steroids. It is not recommended, however, for sugars. The method is based upon the use of BSA in an uncatalyzed reaction. No trimethylchlorosilane should be used in this reaction. Hydrochlorides should be avoided because HCl also will act as a catalyst.

1. Combine 0.1-5.0 mg of sample and 0.2-0.4 ml Pierce BSA in a 1.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial. If material is not soluble in BSA, add 0.1-0.2 ml pyridine.
2. Cap vial and shake for 30 seconds.
3. Heat at 60°C to ease dissolution, if desired.

**NOTE:** Material is silylated at room temperature within times varying from a few minutes to a few hours. Heating will hasten reaction.


**References**

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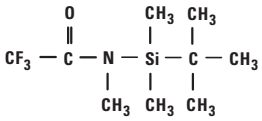
## Ordering Information

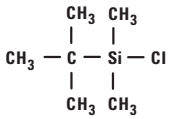
| Product # | Description | Pkg. Size |
|---|---|---|
| **38836** | BSA [*N,O*-bis(trimethylsilyl)acetamide] | 10 x 1 ml ampules |
| **38838** | BSA | 25 g Hypo-Vial Sample Storage Vial |
| **38839** | BSA | 100 g Hypo-Vial Sample Storage Vial |

## MTBSTFA and MTBSTFA + 1% TBDMCS

*Offers stable TBDMS (tert-butyldimethylsilyl) derivatization.*



**MTBSTFA**
MW 241.3
bp 168-170°C
$d_4^{20}$ 1.121



**TBDMCS**
MW 150.73
bp 125°C

### Highlights:

- Derivatizes hydroxyl, carboxyl, thiol and primary and secondary amines
- Typical yields are >96%
- Provides TBDMS ethers that are 104 times more stable to hydrolysis than TMS ethers[1]
- Reaction byproducts are neutral and volatile
- Derivatives have a high molecular concentration at M-57
- Silylating potential increased by adding 1% TBDMS

Thermo Scientific Pierce *N*-Methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) provides TBDMS derivatives without the disadvantage of earlier reported TBDMS-Cl formulations. Bazan and Knapp have demonstrated the usefulness of MTBSTFA by preparing an improved derivative of 6-keto-prostaglandin F1 for GC-MS.[2]

### PROTOCOL

1. Combine 1-10 mg of sample, 0.1 ml Pierce MTBSTFA and 0.1 ml acetonitrile in a 1.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Cap vial and stand at room temperature 5-20 minutes.
3. Analyze by gas chromatography.

**NOTE:** Other solvents may be used including DMF, pyridine and THF. (DMF is not recommended for primary or secondary amines.)


**References**

1. Mawhinney, T.P., *et al.* (1982). *N*-Methyl-*N*-(*tert*-butyldimethylsilyl) Trifluoroacetamide and related *N-tert*-Butyldimethylsilyl amides as protective silyl donors. *J. Org. Chem.* **47**, 3336-3339.
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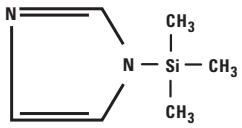
## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 48920 | MTBSTFA [*N*-Methyl-*N*-(*tert*-butyldimethylsilyl) trifluoroacetamide] | 5 ml Hypo-Vial Sample Storage Vial |
| ✖ 48927 | MTBSTFA +1% TBDMS | 10 x 1 ml ampules |

✖*Additional hazardous handling charge.*

## TMSI

*The strongest hydroxyl silylator available for carbohydrates and steroids.*



**TMSI**
MW 140.26
bp 99°C/14 mm Hg
$d_4^{20}$ 0.957

Sakauchi and Horning have shown TMSI to be an all-purpose reagent for unhindered steroids to highly hindered steroids.[2]

Thermo Scientific Pierce TMSI is unique, as it reacts quickly and smoothly with hydroxyls and carboxylic acids, but not with amines.[3,4] Because TMS-derivatives are less stable than TMS-ethers or -esters, TMSI is especially useful in multiderivatization schemes for compounds containing both hydroxyl and amine groups (such as in the preparation of -O-TMS, -N-HFB derivatives of catecholamines).[4]

TMSI is used in the derivatization of alcohols, phenols, organic acids steroids hormones glycols, nucleotides and narcotics. In addition, it is excellent for C1 through C5 fatty acids in serum and urine.[5]

### PROTOCOL 1

This method combines silylation of hydroxyl groups and acylation of amino groups. It was first used by M.G. Horning, *et al.* to prepare catecholamines for GC and GC/MS determinations.[4] This method takes advantage of the fact that TMSI will silylate only hydroxyl groups. Effectively, this blocks those sites from acylation while leaving the amine sites open for acylation.

1. Combine and dissolve 1.0 mg sample and 1.0 ml acetonitrile in a 1.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Add 0.2 ml Pierce TMSI.
3. Cap vial and heat at 60°C for 3 hours.
4. Add 0.1 ml HFBI, TEAI or PFPI (depending on which acyl derivative is desired).
5. Cap vial and heat at 60°C for 30 minutes.
6. Analyze by gas chromatography.

### PROTOCOL 2

This method was developed by Sakauchi and Horning for the silylation of hydroxyl groups on highly hindered steroids. It offers fast conversion to TMS-ethers at a moderate temperature with a single reagent.

1. Combine 0.1-5.0 mg of steroid, 0.1-1.0 ml Pierce TMSI (0.1 ml pyridine should be added for solubilization of cortol and cortolones) in a 1.0 or 3.0 ml Reacti-Vial Small Reaction Vial.
2. Cap vial and heat at 100°C for 2 hours.
3. Analyze by gas chromatography.

### PROTOCOL 3

1. Combine 400 µl Pierce TMSI and 800 µl pyridine (other solvents may be used) in a 3.0 ml Reacti-Vial Small Reaction Vial.
2. Add 10-15 mg sample.
3. Cap vial and shake until sample is dissolved. Heat to 60-70°C if needed.
4. Analyze by gas chromatography.

**NOTE:** TMSI may be used straight with carbohydrates or as a 50% solution with pyridine for wet sugars.


**References**

1. Brittain, G.D. and Schewe, L. (1971). *Recent Advances in Gas Chromatography.* Domsky, I.I. and Perry, J.A. Eds. Marcel Dekker, Inc., New York, NY.
2. Sakauchi, N. and Horning, E.C. (1971). *Anal. Lett.* **4(1)**, 41-52.
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12. Pokushalova, T.V., *et al.* (1985). *J. Chromatogr.* **329**, 189-192.
13. Pokushalova, T.V., *et al.* (1986). Plenum Publishing Corporation.
14. Andrews, P.A., *et al.* (1987). *J. Chromatogr.* **419**, 271-274.
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16. Yoo, Y., *et al.* (1995). Determination of nalbuphine in drug abusers' urine. *J. Anal. Toxicol.* **19,** 120.
17. Seidel, V., *et al.* (1993). Analysis of trace levels of trichothecene mycotoxins in Austrian cereals by gas chromatography with electron capture detection. *Chromatographia* **37,** 191.

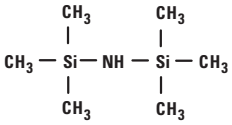

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 88623 | TMSI (*N*-Trimethylsilylimidazole) | 10 x 1 ml ampules |
| 88625 | TMSI | 25 g Hypo-Vial Sample Storage Vial |
| ✖ 88626 | TMSI | 100 g Hypo-Vial Sample Storage Vial |

✖*Additional hazardous handling charge.*

## HMDS

*The popular choice for silylation of sugars and related substances.*[1]



**HMDS**
MW 161.4
bp 125°C
$n_d^{20}$ 1.4071

Thermo Scientific Pierce HMDS greatly extends the practical range of GC, improving chromatographic results in the silylation of sugars and related substances.

A critical study of the optimal proportions of HMDS and trimethylchlorosilane for producing maximum yield of trimethylsilyl derivatives was conducted by Sweeley, *et al.*[1]

### PROTOCOL 1

This protocol describes the method of Sweeley, *et al.* for the trimethylsilylation of sugars and related substances.[1]

1. Combine 10 mg or less carbohydrate sample, 1.0 ml anhydrous pyridine, 0.2 ml Pierce HMDS and 0.1 ml TMCS in a 3.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Cap vial and shake vigorously 30 seconds.
3. Let stand at room temperature 5 minutes or until derivatization is complete.
4. Analyze by gas chromatography.

**NOTE:** Solution may become cloudy when TMCS is added, due to fine precipitate of ammonium chloride. Precipitate will not interfere with gas chromatography. Carbohydrates may be warmed for 10-20 minutes at 75-85°C to hasten dissolution.

### PROTOCOL 2

This method was developed primarily for silylating syrups and concentrated aqueous solutions of sugars such as starch hydrolyzates.[2]

CAUTION: Considerable heat, ammonia gas and pressure emit during reaction. Do not premix.

1. Place 60-70 mg of 80% solids syrup in 3.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Add 1.0 ml pyridine and dissolve.
3. Add 0.9 ml Pierce HMDS and mix.
4. Add 0.1 ml trifluoroacetic acid.
5. Shake vigorously 30 seconds.
6. Let stand 15 minutes.
7. Analyze by gas chromatography.

**References**

1. Sweeley, C.C., *et al.* (1963). *JACS* **85**, 2497-2507.
2. Brobst, K.M. and Lott, C.E., Jr. (1966). *Cereal Chem.* **43**, 35-43.
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5. Jaddou, H., *et al.* (1980). Gas-liquid chromatography of trimethylsilyl derivatives of sugars from Iraqi dates. *J. Agric. Food Chem.* **28**, 1208-1212.
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13. Mahmud, F., *et al.* (1986). Analysis of trimethylsilyl derivatives of sugars derived from the hydrolysates of wood pulp, shoulder material and unfractionated cellulose triacetate by gas liquid chromatography - Part II. *Pakistan J. Sci. Ind. Res.* **29(1)**.
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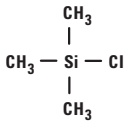
## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 84770 | HMDS (Hexamethyldisilazane) | 25 g Hypo-Vial Sample Storage Vial |
| ✖ 84769 | HMDS | 100 g Hypo-Vial Sample Storage Vial |

✖*Additional hazardous handling charge.*

## TMCS

*An excellent catalyst for difficult-to-silylate compounds.*



**TMCS**
MW 108.7
bp 57.6°C
$d_4^{20}$ 0.858

Thermo Scientific Pierce TMCS (trimethylchlorosilane) provides an excellent adjunct for forming trimethylsilyl ethers for GC determinations.[1,2,3] In addition, it is used for preparing TMS derivatives of organic acids.[4,5]

### PROTOCOL

This protocol describes the method of Sweeley, *et al.* for the trimethylsilylation of sugars and related substances.[1]

1. Combine 10 mg or less carbohydrate sample, 1.0 ml anhydrous pyridine, 0.2 ml HMDS and 0.1 ml Pierce TMCS in a 3.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Cap vial and shake vigorously 30 seconds.
3. Let stand at room temperature 5 minutes or until derivatization is complete.
4. Analyze by gas chromatography.

**NOTE:** Solution may become cloudy when TMCS is added due to fine precipitate of ammonium chloride. Precipitate will not interfere with gas chromatography. Carbohydrates may be warmed for 10-20 minutes at 75-85°C to hasten dissolution.


**References**

1. Sweeley, C.C., *et al.* (1963). *JACS* **85**, 2497-2507.
2. Hedgley, E.V. and Overend, W.G. (1960). *Chem. and Ind. (London).* **378**.
3. Makita, M. and Wells, W.W. (1963). *Anal. Biochem.* **5**, 523.
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5. Mehrota, R.C. and Pant, B.C. (1963). *J. Ind. Chem. Soc.* **40**, 623.
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## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 88530 | TMCS | 25 g bottle |

✖*Additional hazardous handling charge.*

## Methoxamine (MOX) Reagent

*Use this reagent for preparing oximes of steroids and ketoacids prior to silylation.*

Thermo Scientific Pierce MOX Reagent (M.W. 83.51) converts keto groups to methoxime derivatives. It prevents the formation of multiple derivatives (which interfere with quantitation) when enols are present during silylation. Our MOX Reagent is a 2% solution of methoxyamine•HCl in pyridine, and it is used primarily with steroids.

The procedures below are used to prepare methoxime derivatives of steroids and ketoacids prior to silylation. Forming methoximes is based on the work of Fates and Luukkainen, with further applications by Horning, *et al.* Both procedures have been used successfully by Horning, *et al.*[1]

### PROTOCOL 1

This simplified procedure is for stable ketones that are readily soluble in organic solvent.

1. Combine 2 mg sample and 0.5 ml Pierce MOX Reagent in a 10 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Cap vial and heat at 60°C for 3 hours.
3. Add 2 ml water.
4. Extract with three 5 ml portions of high-purity benzene.
5. Combine benzene extracts and wash with 1 N HCl, followed by bicarbonate solution.
6. Dry over anhydrous magnesium sulfate and evaporate to 0.5 ml with nitrogen.
7. Analyze by gas or thin layer chromatography.

### PROTOCOL 2

This procedure is for polar steroids, such as corticoids, that have several hydroxyl groups.[2]

1. Combine 2 mg sample and 0.5 ml Pierce MOX Reagent in a 10 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Cap vial and let stand overnight at room temperature.
3. Add 2 ml saturated NaCl solution.
4. Extract with three 5 ml portions of high-purity ethyl acetate.
5. Combine ethyl acetate extracts and wash with salt saturated 0.1 N HCl wash, followed by a 5% $NaHCO_3$ salt saturated wash.
6. Dry over anhydrous magnesium sulfate and evaporate with nitrogen to 0.5 ml.
7. Analyze by gas or thin layer chromatography.

**NOTE:** After completing the methoxime reaction, some researchers have silylated the reacted mixture without further treatment. The resulting mixture was centrifuged to remove solids, and aliquots of the sample were used for gas chromatography.

**References**

1. Horning, M.G., *et al.* (1968). *Anal. Biochem.* **22**, 284.
2. Maume, B., *et al.* (1968). *Anal. Lett.* **1**, 401.
3. Laine, R.A., *et al.* (1971). Analysis of trimethylsilyl-*O*-methyloximes of carbohydrates by combined gas-liquid chromatography-mass spectrometry. *Anal. Biochem.* **43**, 533-538.
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## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 45950 | Methoxamine (MOX) Reagent (2% methoxyamine•HCl in pyridine) | 10 ml Hypo-Vial Sample Storage Vial |

✖*Additional hazardous handling charge.*

## Tri-Sil HTP (HDMS: TCMS: pyridine) Reagent

*Our reagent-catalyst solvent mixture for one-step derivatization.*

Thermo Scientific Pierce Tri-Sil HTP Reagent is composed of HMDS, TMCS and high purity pyridine. It is useful for rapid production of TMS derivatives of polar compounds for gas chromatographic determination and biochemical synthesis. The Tri-Sil HTP Reagent is ideal for GC determinations of:

- Sugars[1-6]
- Alcohols[1]
- Phenols[7]
- Steroids[8,9]
- Sterols[10,11]
- Bile acids and other organic acids[12-14]
- Some amines[15-17]

Our Tri-Sil HTP Reagent is based on the procedure of Sweeley, *et al.*[1] and is used for the optimal conversion of organic hydroxyl and polyhydroxyl compounds into TMS ethers. The reaction proceeds as:

$$3\ ROH + Me_3SiNHSiMe_3$$
$$Me_3SiCl - 3\ ROSiMe_3 + NH_4Cl$$

The versatility, speed and ease of use of our Tri-Sil HTP Reagent has made it the most widely used silylation formulation available.

### PROTOCOL

1. Combine 5-10 mg sample and 1.0 ml Pierce Tri-Sil HTP Reagent in a 3.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Shake the reaction vigorously for 30 seconds or warm to 75-85°C to dissolve.
3. React at room temperature for 5 minutes.
4. Analyze by gas chromatography.

**NOTE:** A majority of hydroxyl and polyhydroxyl compounds will be completely derivatized in less than 5 minutes including sugars, phenols, organic acids, some amines and alcohols. Highly hindered compounds, such as some steroids, may require 15 minutes to 8 hours. Extremely intractable compounds may require refluxing for several hours.


**References**

1. Sweeley, C.C., *et al.* (1963). *JACS* **85**, 2497-2507.
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13. Burkhard, C.A. (1957). *J. Org. Chem.* **22**, 592.
14. Mehrotra, R.C. and Pant, B.C. (1963). *J. Ind. Chem. Soc.* **40**, 623.
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19. Ng, L., *et al.* (1993). Simple gas chromatographic method for the assay of salts of carboxylic acids as their trimethylsilyl derivatives. *J. Chromatogr.* **637,** 104.


## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 48999 | Tri-Sil HTP Reagent HMDS:TCMS: Pyridine (2:1:10) | 10 x 1 ml ampules |
| ✖ 49001 | Tri-Sil HTP Reagent HMDS: TCMS: Pyridine (2:1:10) | 50 ml Hypo-Vial Sample Storage Vial |

✖*Additional hazardous handling charge.*

## Tri-Sil BP Reagent (BSA:pyridine)

*A reagent-solvent solution for one-step derivatization.*

Thermo Scientific Pierce Tri-Sil BP Reagent is composed of BSA (2.5 mEq/ml*) and Pyridine.

**1.25 mEq for amides*

Tri-Sil BP Reagent reacts with:

- Alcohols phenols, some enols and other hydroxyl and polyhydroxyl compounds to form trimethylsilyl ethers
- Organic acids to form trimethylsilyl esters
- Aromatic amides to form N-trimethylsilyl derivatives
- Amines to form N-trimethylsilyl derivatives

In addition, Tri-Sil BP Reagent is excellent for unhindered steroids, but it is not recommended for carbohydrates.

### PROTOCOL

1. Combine 5-10 mg sample and 1.0 ml Pierce Tri-Sil BP Reagent in a 3.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Cap vial and heat at 60-70°C for 15-20 minutes to facilitate dissolution and derivatization.
3. Analyze by gas chromatography.

**References**

1. Fennessey, P.V., *et al.* (1980). Rearrangements of the TMS derivatives of acylglycines. *Org. Mass Spec.* **15(4).**
2. Ramsdell, H.S., *et al.* (1980). Gas chromatographic retention indices of twenty metabolically important acylglycines as trimethylsilyl derivatives. *J. Chromatogr.* **181**, 90-94.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 49012 | Tri-Sil BP Reagent (2.5 mEq/ml BSA in pyridine) | 25 ml Hypo-Vial Sample Storage Vial |

✖*Additional hazardous handling charge.*

## Tri-Sil TBT Reagent (TMSI:BSA:TCMS)

*A powerful catalyzed silylation reagent formulation.*

Thermo Scientific Pierce Tri-Sil TBT is a mixture containing three parts TMSI, three parts BSA and two parts TMCS. Our Tri-Sil TBT Reagent converts all classes of hydroxyl groups to TMS ethers. Under usual conditions, the reaction is complete in a short period of time at 60-80°C. Highly hindered hydroxyls may require several hours.

### PROTOCOL

This method is used to silylate all hydroxyl groups in steroid structures, even the most sterically hindered, such as the 17 hydroxyl groups in cortol. This method also has been used by Bacon and Kokenakes to measure plasma prednisolone by GC.

1. Combine 0.1-5.0 mg sample and 0.2-0.4 ml Pierce Tri-Sil TBT Reagent in a 1.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Cap vial and shake to dissolve.
3. Heat at 60-80°C for 6-24 hours to complete reaction.
4. Analyze by gas chromatography.

**NOTE:** If sample is insoluble, add 0.1-0.2 ml pyridine.

**References**

1. Huizing, H.J., *et al.* (1986). Positive and negative ion chemical ionization mass spectrometry of trimethylsilyl derivatives of pyrrolizidine alkaloids using $NH^+$ or OH as the reactant ions. *Biomed. and Environ. Mass Spec.* **13**, 293-298.
2. Seidel, V., *et al.* (1993). Analysis of trace levels of trichothecene mycotoxins in Austrian cereals by gas chromatography with election capture detection. *Chromatographia* **37**, 191.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 49016 | Tri-Sil TBT Reagent TMSI: BSA: TMCS (3:3:2) | 10 x 1 ml ampule |

✖*Additional hazardous handling charge.*

## Tri-Sil TP Reagent (TMSI:pyridine)

*Great for derivatizing hydroxyl compounds.*

Thermo Scientific Pierce Tri-Sil TP Reagent is a mixture of TMSI in dry pyridine (1.5 mEq/ml). It is used primarily for derivatizing hydroxyl compounds, particularly carbohydrates. Tri-Sil TP Reagent has been used successfully for the silylation of alcohols and phenols, organic acids, hydroxylamines, amino acids, carbohydrates, flavonoids, glycols and polyglycols, nucleotides, steroids, hydroxyl acids, barbiturates, narcotics, indoles, and vitamins. Tri-Sil ZTP Reagent does not react with amines.

Our Tri-Sil TP Reagent can be used in the presence of water as long as there is enough reagent present to react with both the water and the sample. The reagent reads with water in a 2:1 ratio.

### PROTOCOL 1

1. Combine 10-15 mg sample and 1.0 ml Pierce Tri-Sil TP Reagent in a 3.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Cap vial and shake to dissolve. If necessary, heat at 60-70°C. Silylation is complete upon dissolution.
3. Analyze by gas chromatography.

### PROTOCOL 2

For solutions containing ~1% or less total sugars, use a 50:50 v/v TMSI/pyridine solution.

1. Evaporate ~50 µl sugar solution to a glassy syrup in a 0.3 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Add 50 µl Pierce Tri-Sil TP Reagent.
3. Cap vial and heat at 60°C to dissolve and derivatize the sugars.
4. Analyze directly by gas chromatography.


**References**

1. Hymowitz, T., *et al.* (1972). Relationship between the content of oil, protein and sugar in soybean seed. *Agronomy J.* **64,** 613-616.
2. Mamer, 0. and Gibbs, B. (1973). *Clin. Chem.* **19(9)**, 1006-1009.
3. Quilliam, M.A., *et al.* (1980). Study of rearrangement reactions occurring during gas chromatography of *tert*-butyldimethylsilyl ether derivatives of uridine. *J. Chromatogr.* **194**, 379-386.
4. Low, N., *et al.* (1994). Normative data for commercial pineapple juice from concentrate. *J. of AOAC International* **77,** 965.


## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 49230 | Trl-Sil TP Reagent TMSI: Pyridine (1:4) | 10 x 1 ml ampules |
| ✖ 49231 | Trl-Sil TP Reagent TMSI: Pyridine (1:4) | 25 ml Hypo-Vial Sample Storage Vial |

✖*Additional hazardous handling charge.*

## Silylation Grade Solvents

*Manufactured to meet your exacting silylation needs.*

Thermo Scientific Pierce Silylation Grade Solvents are specially manufactured and packaged to meet the exacting needs of silylation and other sensitive derivatization reactions. Each Silylation Grade Solvent is purified, dried and packaged under nitrogen in our convenient Hypo-Vial Sample Storage Vials. Supplied complete with elastomer septa, this unique packaging allows immediate access to your sample, without exposure to moisture and oxygen.

### Highlights:

- Purified, dried and packaged under nitrogen in convenient Hypo-Vial Sample Storage Vials
- Supplied with elastomer septa, allowing immediate access to sample without exposure to moisture and oxygen
- Use polar solvents (acetonitrile, dimethylformamide, dimethylsulfoxide, pyridine and tetrahydrofuran) to facilitate reactions; nonpolar organic solvent may be used, but they will not accelerate the rate of reaction
- Avoid water or alcohol because TMS reagents react with active hydrogen; avoid enolizable ketones
- Use dimethylformamide for steroids and other large molecules
- Use dimethylsulfoxide to prepare TMS derivatives of tertiary alcohols and some compound with reluctant solubility in other silylation solvents
- Pyridine is an excellent solvent and reaction medium for MS reactions and is an HCl acceptor in reactions involving organochlorosilanes
- Other commonly used solvents include tetrahydrofuran and acetonitrile

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 20062 | Acetonitrile | 50 ml Hypo-Vial Sample Storage Vial |
| ✖ 20672 | Dimethylformamide | 50 ml Hypo-Vial Sample Storage Vial |
| 20684 | Dimethylsulfoxide | 50 ml Hypo-Vial Sample Storage Vial |
| ✖ 27530 | Pyridine | 50 ml Hypo-Vial Sample Storage Vial |
| ✖ 27860 | Tetrahydrofuran | 50 ml Hypo-Vial Sample Storage Vial |

✖*Additional hazardous handling charge.*
*For HPLC Grade Solvents, see page 35, 53.*

# Introduction to Thermo Scientific Pierce **Acylation Reagents**

Acylation is the conversion of compounds (through the action of a carboxylic acid or a carboxylic acid derivative) that contain active hydrogens such as -OH, -SH and -NH esters; thioesters; and amides.[1] In chromatographic applications, the acylation reaction is used primarily for converting the above classes of compounds into derivatives that are better suited for chromatography[2] or that give a greater response to the chromatographic detection system than the parent compound.[3]

An important example of this application is the insertion of perfluoroacyl groups into a molecule to enhance the detectability of the substance by electron capture. The presence of a carbonyl group adjacent to the halogenated carbons enhances the electron capture detector (ECD) response.

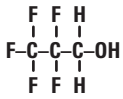
Acyl derivatives also are useful in MS applications in which they direct the fragmentation patterns of the compounds to be studied.[4]


**References**

1. Donike, M. (1973). Acylation with bis (acylamides). *N*-Methyl-bis (trifluoroacetamide), two new reagents for trifluoroacetylation. *J. Chromatogr.* **78**, 273-279.
2. Sullivan, J.E. and Schewe, L.R. (1977). Preparation and gas chromatography of highly volatile trifluoroacetylated carbohydrates using *N*-Methyl-bis (trifluoroacetamide). *J. Chromatogr. Sci.* **15**, 196-197.
3. Benington, F., *et al.* (1975). Identification and separation of indolealkylamines by gas liquid chromatographic analysis of their heptafluorobutyryl derivatives. *J. Chromatogr.* **106**, 435-439.
4. Borga, O., *et al.* (1971). Quantitative determination of nortriptyline and desmethylnortriptyline in human plasma by combined gas chromatography-mass spectrometry. *J. Chromatogr.* **4(12)**, 837-849.


## Pentafluoropropanol

*Purified for GC/MS use.*



**Pentafluoropropanol**
MW 150.05
bp 80.6°C
$d_4^{20}$ 1.2880

### Highlights:

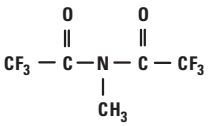
- Addition of fluorine atoms into compounds greatly enhances the sensitivity of certain detectors for those materials
- It is advantageous to introduce fluorine atoms for ECD and GC/MS applications
- Carboxylic acids can be derivatized using a two-step reaction involving reaction with an anhydride, followed by a fluorinated alcohol

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 65195 | Pentafluoropropanol | 10 x 1 ml ampules |

## MBTFA

*For trifluoroacylating primary and secondary amines, hydroxyl and thiol groups, and carbohydrates.*



**MBTFA**
MW 223.08
bp 123-124°C
$d_4^{20}$ 1.55

Thermo Scientific Pierce MBTFA is ideal for trifluoroacylating primary and secondary amines, hydroxyl and thiol groups, or carbohydrates under nonacidic conditions.[1] In addition, MBTFA is used to selectively acylate amines in the presence of hydroxyl and carboxyl functions. The reaction byproduct, *N*-methyltrifluoroacetamide, is volatile. MBTFA also produces very volatile derivatives of carbohydrates.[2]

### Highlights:

- Trifluoroacetylates primary and secondary amines, as well as hydroxyl and thiol groups under mild nonacidic conditions
- Principle byproduct from the derivatization reaction is *N*-methyltrifluoroacetamide, which is stable, volatile and does not present problems in subsequent GC
- Produces very volatile derivatives of carbohydrates and can be used to selectively acylate amines in the presence of hydroxyl and carboxyl groups that have been protected by silylation

### PROTOCOL 1

For trifluoroacylating primary and secondary amines, and hydroxyl and thiol groups.

1. Combine 1-10 mg sample and 0.1-0.2 ml Pierce MBTFA. If sample is not easily solubilized, add 0.5-1.0 ml pyridine, DMF, THF or acetonitrile. (MBTFA can be pre-mixed with solvent in a 1:4 ratio. Add 1 ml pre-mixed solution to 1-10 mg compound.)
2. Cap vial and heat at 60-100°C for 10-30 minutes (longer for hindered compounds).
3. Cool and analyze by gas chromatography.

**NOTE:** MBTFA reacts with amines at room temperature to yield quantitative results in approximately 30 minutes. Hydroxyl groups are slower to react. As a general procedure, reaction mixtures should be heated 10-30 minutes at 60-100°C. In the case of hindered compounds, further heating may be necessary.

### PROTOCOL 2

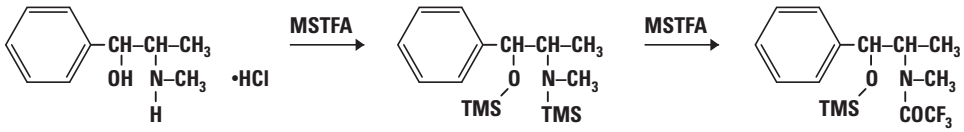
For trifluoroacylating sugars.[2]

Producing TFA derivatives of sugars using standard fluorinated anhydride and fluorinated acylimidazole procedures has yielded multiple or unstable derivatives. MBTFA produces the corresponding trifluoroacetyl derivatives of the mono-, di-, tri- and tetrasaccharides. These derivatives, when subjected to gas chromatography, produce quantitative results and yielded an unexpectedly high degree of volatility.

The high volatility of the corresponding TFA derivatives yields shorter retention times at lower temperatures than other commonly used silylation methods. The result is that polar columns with lower maximum temperature limits can be used to provide faster separations under less stringent chromatographic conditions. Mixtures of carbohydrates containing mono- through tetrasaccharides can be analyzed in a single run in as little as 15 minutes.

1. Combine 5-10 mg dry sugar and 0.5 ml Pierce MBTFA in a 5 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Add 0.5 ml pyridine.
3. Cap vial and heat at 65°C for 1 hour with occasional shaking.
4. Analyze 1 µl by gas chromatography.

**NOTE:** Reactions are complete upon dissolution.




**Selective acylation of amine groups in the presence of hydroxyl and carboxyl groups** is possible if these groups are first protected by silylation. The multifunctional compound first is silylated with MSTFA (*N*-Methyl-*N*[TMS] trifluoroacetamide), which silylates all of the functional groups (TMS is trimethylsilyl). The compound then is further reacted with MBTFA, exchanging the TMS-group on the amino function with a trifluoroacetyl group. Similar results are obtained with amino acids that yield *N*-Trifluoroacetyl-O-TMS-esters.

## Ordering Information

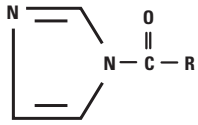
| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 49700 | MBTFA [N-Methyl-bis(trifluoroacetamide)] | 10 x 1 ml ampules |
| ✖ 49701 | MBTFA | 5 g bottle |
| ✖ 49703 | MBTFA | 25 ml |
| ✖ 49704 | MBTFA | 100 ml |

✖ *Additional hazardous handling charge.*

**References**
1. Donike, M. (1973). *N*-Methyl-bis (trifluoroacetamid) und bis (trifluoroacetamide), ZWEI Neue Reagenzien. Zurtrifluoroacetylieerung. *J. Chromatogr.* **78**, 273-279.
2. Sullivan, J., *et al.* (1977). Volatile trifluoroacetylated carbohydrates using *N*-Methyl *bis*(Trifluoroacetamide). *J. Chromatogr. Sci.* **15**, 196-197.
3. Weitz, C.J., *et al.* (1986). Morphine and codeine from mammalian brain. *Proc. Nat. Acad. Sci. USA.* **83**, 9784-9788.

## Perfluoroacylimidazoles (TFAI[1-5] and HFBI[6-9])

*Offer effective acylation of hydroxyl groups and primary and secondary amines.*




| R | Name | M.W. | b.p. | $d_4^{20}$ |
|---|---|---|---|---|
| $CF_3$ | TFAI | 164.08 | 38-40°C/14 mm | 1.490 |
| $C_3F_7$ | HFBI | 264.10 | 57-58°C/10 mm | 1.562 |

In many cases, the use of *N*-acylimidazoles offers considerable advantages over acid chlorides and anhydrides. Advantages include:

### Highlights:

- The reaction is smooth and positive, releasing no acids into the system to hydrolyze samples
- The byproduct, imidazole, is relatively inert
- Ideal for FID and ECD techniques
- Derivatives are volatile, despite size of group
- Closely bound fluorines contribute stability

Fluorinated acylimidazoles acylate hydroxyl groups and primary and secondary amines. They react smoothly with indole alkyl-amines to quantitatively derivatize the indole nitrogen, as well as other functional groups present.[1,2] Fluorinated imidazoles also are used for bifunctional derivatizations and in exchange reactions from the TMS derivative to the HFB derivative. In addition, a study by Ikekawa and colleagues found that *O*-TMS groups could be exchanged to *O*-HFB groups by adding HFBI and a small amount of HFB acid directly to the reaction mixture.[3,4]

### PROTOCOL 1

Preparing fluoro acyl derivatives for FID.

1. Place 0.1-2.0 mg sample in a 1.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Add 0.2 ml desired Pierce *N*-acylimidazole.
3. Cap vial and heat at 60°C 15-30 minutes, or until reaction is complete. (Moderately and hindered steroids may require 2-6 hours heating.)
4. Analyze by FID gas chromatography.

### PROTOCOL 2

Preparing HFB derivatives of indolealkyl amines using HFBI for FID and ECD techniques.[2]

Milligram-scale Derivatization:

1. Combine 2 mg sample and 0.2 ml Pierce HFBI in 3 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Cap vial and heat at 85°C for 1 hour.
3. Add 1 ml water and 2 ml toluene.
4. Cap vial and shake 2 minutes.
5. Analyze toluene layer by gas chromatography.

**NOTE:** A small amount of HFB acid remains in toluene phase. If it interferes with analysis, wash toluene phase with 2-3 more 0.5 ml water washes.

Microgram-Scale Derivatization:

1. Combine 2 µg to pg quantities residue and 20 µl Pierce HFBI in 3.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Cap vial and heat at 85°C for 1 hour.
3. Add 2 ml pure toluene and 0.5 ml distilled water.
4. Cap vial and shake 2 minutes.
5. Remove aqueous layer.
6. Wash toluene layer 3 times with 0.5 ml water.
7. Centrifuge toluene layer 2 minutes.
8. Analyze toluene layer by GC using ECD.

**References**

1. Seeley, S.D. and Powell, L.D. (1974). *Anal. Biochem.* **58**, 39-46.
2. Bennington, F., *et al.* (1975). *J. Chromatogr.* **106**, 435-439.
3. Ikekawa, N., *et al.* (1972). *J. Chrom Sci.* **10**, 233-242.
4. Miyazaki, H., *et al.* (1973) *Anal. Chem.* **45(7)**, 1164-1168.
5. Horning, M.G., *et al.* (1968). *Anal. Lett.* **1**, 311-321.
6. Mayhew, J.W., *et al.* (1978). Gas-liquid chromatographic method for the assay of aminoglycoside antibiotics in serum. *J. Chromatogr.* **151**, 133-146.
7. Cohen, H., *et al.* (1984). Capillary gas chromatographic determination of T-2 toxin, HT-2 toxin and diacetoxyscirpenol in cereal grains. *J. Ass. Off Anal. Chem.* **67(6).**
8. Krishnamurthy, T., *et al.* (1987). Mass spectral investigations of trichothecene mycotoxins. II. Detection and quantitation of macrocyclic trichothecenes by gas chromatography/negative ion chemical ionization mass spectrometry. *J. Ass. Off Anal. Chem.* **70(1).**
9. Quilliam, M.A., *et al.* (1980). Study of rearrangement reactions occurring during gas chromatography of *tert*-butyldimethylsilyl ether derivatives of uridine. *J. Chromatogr.* **194**, 379-386.

## Ordering Information

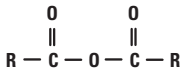
| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 48882 | TFAI (Trifluoroacetylimidazole) | 10 x 1 ml ampules |
| ❄ 44211 | HFBI (Heptafluorobutyrylimidazole) | 5 g bottle |

✖*Additional hazardous handling charge.*

❄*Additional dry ice and/or freight charges.*

## Perfluoro Acid Anhydride (TFAA, PFAA and HFAA)

*Ours are high-purity, ideal for preparing fluoracyl derivatives.*



| R | Name | M.W. | b.p. | $d_4^{20}$ |
|---|---|---|---|---|
| $CF_3$ | TFAA | 210.0 | 39.5-40.5°C | 1.490 |
| $C_2F_5$ | PFAA | 310.0 | 74°C | 1.571 |
| $C_3F_7$ | HFAA | 410.0 | 106-107°C | 1.665 |

Fluorinated anhydrides are used to prepare fluoracyl derivatives for GC/MS, they produce stable volatile derivatives for FID and ECD techniques.

### PROTOCOL 1

Preparing fluoracyl derivatives of amines and alcoholic compounds on a submicrogram scale for ECD.

1. Combine < 50 ng sample dissolved in 500 µl benzene and 100 µl 0.05 M TEA in benzene in a 5.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Add 10 µl Pierce Acid Anhydride.
3. Cap vial, heat at 50°C 15 minutes, then cool.
4. Add 1 ml water, cap vial and shake 1 minute.
5. Add 1 ml 5% aqueous ammonia, cap vial and shake 5 minutes.
6. Centrifuge.
7. Inject 1-10 µl benzene phase for ECD.

**NOTE:** Use 250 µg for FID. Excess TEA is required for quantitative conversion of amines. TEA does cause disturbances in the chromatogram at high EC sensitivity. The benzene used as sample solvent and TEA solvent should be dry, as water will compete for the anhydride during reactions. The amount of anhydride used in the procedure (10 µl) is 25% more than necessary for a complete reaction - even if the 0.5 ml benzene used is water-saturated.

### PROTOCOL 2

Preparing fluoracyl derivatives of phenols for FID and ECD.

For Flame Ionization Detection:

1. Combine 1 mg sample dissolved in 0.5 ml benzene and 200 µl 0.1 M TEA in benzene.
2. Add 25 µl Pierce Acid Anhydride.
3. Cap vial and let react at room temperature for 15 minutes.
4. Add 0.5 ml 1 M phosphate buffer, pH 6.0, and shake 30 seconds.
5. Centrifuge.
6. Separate organic phase. Analyze by GC.

For Electron Capture Detection:

1. Combine 0.5 ml benzene containing the sample and 10 µl TEA in benzene in 5 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Add 10 µl Pierce HFAA.
3. Cap vial and let react at room temperature for 10 minutes.
4. Add 0.5 ml 1 M phosphate buffer, pH 6.0, and shake 30 seconds.
5. Centrifuge; analyze 2 µl benzene phase by GC.

**NOTE:** Excess anhydride and acid are removed by the aqueous extraction. No ECD disturbances from the water or other constituents. HFB-esters of phenols are stable in the presence of water (with aqueous phase at pH ≤ 6.0). Alkaline extraction with reagents, such as aqueous ammonia, decomposes the HFB ester. A pH ≤ 6.0 maintains the TEA catalyst in the protonized form. TEA in the unprotonized form will catalyze decomposition of the esters.

**References**

1. Walle, T. and Ehrsson, H. (1970). *Acta Pharm. Suecica* **7**, 389-406.
2. Walle, T. and Ehrsson, H. (1971). *Acta Pharm. Suecica* **8**, 27-38.
3. Ehrsson, H., *et al.* (1971). *Acta Pharm. Suecica* **8**, 319-328.
4. Suzuki, S., *et al.* (1983). Rapid screening method for methamphetamine in urine by colour reaction in a Sep-Pak $C_{18}$ cartridge. *J. Chromatogr.* **267**, 381-387. (TFAA)
5. Seifert, W.E., *et al.* (1978). Characterization of mixtures of dipeptides by gas chromatography/mass spectrometry. *Anal. Biochem.* **88**, 149-161. (PFAA)
6. Christophersen, A.S., *et al.* (1987). Identification of opiates in urine by capillary column gas chromatography of two different derivatives. *J. Chromatogr.* **422**, 117-124. (PFAA)
7. Mule, S.J., *et al.* (1988). Confirmation of marijuana, cocaine, morphine, codeine, amphetamines, methamphetamines, phencyclidine by GC/MS in urine following immuno-assay screening. *J. Anal. Tox.* **12**, 102-107. (PFAA)

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 67363 | TFAA (Trifluoroacetic Acid Anhydride) | 100 g bottle |
| ✖ 65193 | PFAA (Pentafluoropropionic Acid Anhydride) | 10 x 1 ml ampules |
| ✖ 65192 | PFAA | 25 g bottle |
| ✖ 65191 | PFAA | 100 g bottle |
| ✖ 63164 | HFAA | 10 x 1 ml ampules |
| ✖ 63163 | HFAA | 25 g bottle |
| ✖ 63162 | HFAA | 100 g bottle |

✖*Additional hazardous handling charge.*

# Thermo Scientific Pierce **Alkylation Reagents**

When used in derivatization for gas chromatography, alkylation represents the substitution of an active hydrogen by an aliphatic or aliphatic-aromatic[1] (benzyl) group. This technique is used to modify those compounds containing acidic hydrogens, such as carboxylic acids and phenols. The principal chromatographic use of this reaction is the conversion of organic adds into esters, which produce better chromatograms than the free acids.

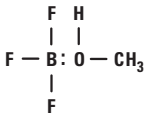
In addition, alkylation reactions can be used to prepare ethers, thioethers and thioesters; *N*-alkylamines; and amides.[2] As the acidity of the active hydrogen decreases, the strength of the alkylating reagent must be increased. As the reagents and conditions become harsher, the selectivity and applicability of the methods become more limited.


**References**

1. Kawahara, F.K. (1968). Microdetermination of derivatives of phenols and mercaptans by means of electron capture gas chromatography. *Anal. Chem.* **40(6)**, 1009.
2. Kananen, G., *et al.* (1972). Barbiturate analysis - a current assessment. *J. Chrom. Sci.* **10**, 283-287.


## $BF_3$-Methanol

*Provides convenient, fast and quantitative esterification of fatty acids.*



**$BF_3$-Methanol**
14% $BF_3$ MW 67.82
86% $CH_3OH$ MW 32.04

$BF_3$-Methanol methylation is one of the most convenient ways to prepare methyl esters of fatty acids. Supplied in an easy-to-use septum-sealed Hypo-Vial Sample Storage Vial, Thermo Scientific Pierce $BF_3$-Methanol offers convenient syringe removal of your sample - without exposing the contents.

### PROTOCOL 1

For preparing fatty acid methyl esters.

1. Combine 100 mg fatty acid and 3 ml Pierce $BF_3$-Methanol in a 5.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Cap vial and heat at 60°C for 5-9 minutes.
3. Cool and transfer to separatory funnel with 30 ml hexane.
4. Wash 2 times with saturated NaCl solution.
5. Discard aqueous layers.
6. Dry over sodium sulfate.
7. Evaporate solvent under nitrogen.
8. Analyze by gas chromatography.

### PROTOCOL 2

For preparing C8-C17 fatty acids.

1. Combine 500 mg fatty acid and 5 ml Pierce $BF_3$-Methanol in a 25 ml flask.
2. Heat on a steam bath 5 minutes.
3. Add saturated NaCl solution until total volume is ~20 ml.
4. Cap flask and invert several times.
5. Allow organic layer to collect at the top, then separate.
6. Dry organic layer over $Na_2SO_4$.
7. Evaporate solvent under nitrogen.
8. Analyze by gas chromatography.


**Reference**

1. Yao, Z., *et al.* (1988). Choline deficiency causes translocation of CTP: phosphocholine cytidylytransferase from cytosol to endoplasmic reticulum in rat liver. *J. Biol. Chem.* **265(8)**, 4326-4331.

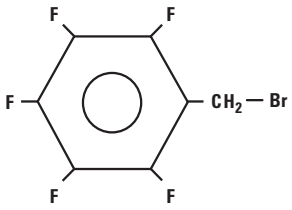

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ **49370** | $BF_3$-Methanol | 100 ml Hypo-Vial Sample Storage Vial |

✖*Additional hazardous handling charge.*

## Pentafluorobenzyl Bromide (PFBBr)

*For the electron capture GC analysis of carboxyl acids, phenols and sulfonamides.*




**PFBBr**
MW 260.9
bp 174-175°C
$d_4^{20}$ 1.86

Pentafluorobenzylation by an "Extraction Alkylation" technique has been described for the electron capture GC analysis of carboxyl acids, phenols and sulfonamides. This process uses tetrabutylammonium as a counter ion and methylene chloride as a solvent. Reaction times are fast (~20 minutes). Derivatives are highly EC-sensitive, making them useful in low-level determinations of fatty acids.

Kawahara performed extensive work with this reagent, using a potassium carbonate catalyst for the electron capture analysis of mercaptans, phenols and organic acids in surface water.[1-3]

PFBBr has been applied in analyzing trace organics in asphalts, as a "fingerprinting" technique for identifying asphalt pollutants found in surface waters.

### PROTOCOL

For pentafluorobenzylation of acids, phenols and sulfonamides.[1,2]

1. Place 1 ml methylene chloride containing 0.2 mg sample in 3 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Add 1 ml aqueous 0.1 M TBA hydrogen sulfate and 0.2 M sodium hydroxide solution.
3. Add 20 µl Pierce PFBBr.
4. Cap vial and shake 20-30 minutes.
5. Inject portion of methylene chloride phase into chromatograph for FID analysis.
6. Evaporate methylene chloride to dryness with nitrogen and redissolve with benzene for ECD analysis.

**References**

1. Kawahara, F.K. (1968). *Anal Chem.* **40(6)**, 1009.
2. Kawahara, F.K. (1968). *Anal Chem.* **40(13)**, 2073.
3. Kawahara, F.K. (1971). Gas chromatographic analysis of mercaptans, phenols and organic acids in surface waters with use of pentafluorobenzyl derivatives. *Environ. Sci. Technol.* **5(3)**.
4. Ehrsson, H. (1971). Quantitative gas chromatographic determination of carboxylic acids and phenols after derivatization with pentafluorobenzyl bromide. *Acta Pharm. Suecica* **8**,113-118.
5. Kawahara, F.K. (1976). *Environ. Sci. Technol.* **10(8)**, 761.
6. Gyllenhaal, 0., *et al.* (1975) Determination of sulphonamides by electron capture gas chromatography. *J. Chromatogr.* **107**, 327-333.
7. Kari, S., *et al.* (1981). Modification of glass capillary gas chromatographic columns by alkylation of the glass surface with pentafluorobenzyl bromide. *Chromatographia* **14(8)**.
8. Lee, H.-B., *et al.* (1984). Chemical derivatization analysis of pesticide residues. IX. Analysis of phenol and 21 chlorinated phenols in natural waters by formation of pentafluorobenzyl ether derivatives. *J. Assoc. Off. Anal. Chem.* **67(6)**.
9. Casper, H.H., *et al.* (1985). Capillary gas chromatographic-mass spectrometric determination of fluoroacetate residues in animal tissues. *J. Assoc. Off. Anal. Chem.* **68(4)**.
10. Kok, R.M., *et al.* (1985). Highly sensitive determination of 5-fuorourcil in human plasma by capillary gas chromatography and negative ion chemical ionization mass spectrometry. *J. Chromatogr.* **343**, 59-66.
11. Odham, G., *et al.* (1985). Determination of microbial fatty acid profiles at femtomolar levels in human urine and the initial marine microfouling community by capillary gas chromatography-chemical ionization mass spectrometry with negative ion detection. *J. Microbiol. Meth.* **3**, 331-344.
12. Rosenfeld, J.M., *et al.* (1986). Solid-supported reagents in the determination of cannabinoids in plasma. *Anal. Chem.* **58**, 716-721.
13. Rosenfeld, J.M., *et al.* (1986). Solid-supported reagents for the simultaneous extraction and derivatization of carboxylic acids from aqueous matrices. *J. Chromatogr.* **358**, 137-146.
14. Vairavamurthy, A., *et al.* (1986). Determination of acrylic acid in aqueous samples by electron capture gas chromatography after extraction with tri-n-octylphosphine oxide and derivatization with pentafluorobenzyl bromide. *Anal. Chem.* **58**, 2684-2687.
15. Chen, S.-H., *et al.* (1987). Simultaneous gas chromatographic determination of iodide, nitrate, sulphide and thiocyanate anions by derivatization with pentafluorobenzyl bromide. *J. Chromatogr.* **396**, 129-137.
16. Bartsch, H., *et al.* (1988). Methods for detecting DNA damaging agents in humans: applications in cancer epidemiology and prevention. *International Agency for Research on Cancer. IARC Scientific Publications* **89**.
17. Bosin, T.R. (1988). Measurement of β-carbolines by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr.* **428**, 229-236.
18. Thio, A.P., *et al.* (1988). In-block derivatization of herbicidal carboxylic acids by pentafluorobenzyl bromide. *Anal. Lett.* 21(2), 477-489.

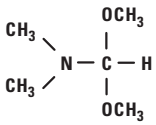
## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 58220 | PFBBr (Pentafluorobenzyl Bromide) | 5 g |

✖*Additional hazardous handling charge.*

## Methylate Reagent (DMFDMA)

*For easy, effective preparation of methyl esters from fatty acids and amino acids.*



**Methylate Reagent**
MW 119.17
bp 102-104°C
$d_4^{20}$ 0.897

For preparing methyl esters for gas chromatography, Thermo Scientific Pierce Methylate Reagent offers significant advantages including:

- **Speed** - the reaction is complete upon dissolution
- **Quantitation** - quantitative yields are obtained when reagent and sample are injected - without prior mixing
- **Your choice of formulation** - our Methylate Reagent is a convenient, ready-to-use reagent that contains 2 mEq/ml in pyridine.

Our Methylate Reagent is stable at room temperature and is packed in convenient, ready-to-use Hypo-Vial Sample Storage Vials. No water washing, extraction or concentration of the derivatives are required. Plus, no water is formed in the reaction.

Reactions with the Methylate Reagent usually are complete upon dissolution, except for long chain solid acids. In these applications, it is necessary to use Methylate Reagent with additional solvent and mild heating. Suitable solvents include pyridine, benzene, methanol, chloroform, methylene chloride, THF and DMF.

Thenot, *et al.* have demonstrated analytical applications that use of the Methylate Reagent for analyzing fatty acids[1] and amino acids.[2]

### PROTOCOL 1

Methods of alkylation using DMF-Dialkyl Acetal Reagents.

1. Combine 50 mg fatty acid and 1 ml Pierce Methylate Reagent in a 3 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Cap vial and heat at 60°C for 10-15 minutes or until dissolution is complete.
3. Analyze by gas chromatography.

### PROTOCOL 2

For preparing *N*-dimethylaminomethylene (DMAM) methyl esters of amino acids.

1. Combine amino acid with 1:1 ratio of Pierce Methylate Reagent to acetonitrile in a Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Cap vial and heat at 100°C for 20 minutes or until dissolution is complete.
3. Analyze by gas chromatography.

**NOTE:** Aspartic acid requires a longer time for complete dissolution. Hydroxyl groups on hydroxyl-substituted amino acids do not react under the above conditions.


**References**

1. Thenot, J.-P., *et al.* (1972). *Anal Lett.* **5(4)**, 217-233.
2. Thenot, J.-P. and Horning, E.C. (1972). Amino Acid *N*-Dimethylaminomethylene Alkyl Ester. *Anal. Lett.* **5(8)**, 519-529.
3. Zhang, Y., *et al.* (1993). Assay of the acetyl-CoA probe acetyl-sulfamethoxazole and of sulfamethoxazole by gas chromatography-mass spectrometry. *Anal. Biochem.* **212,** 481.


## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 49350 | Methylate Reagent (2 mEq/ml in pyridine) (*N,N*-Dimethylformamide dimethyl acetal) | 25 ml Hypo-Vial Sample Storage Vial |

## MethElute Reagent (TMPAH)

*A powerful reagent for accurate, sensitive on-column methylation.*

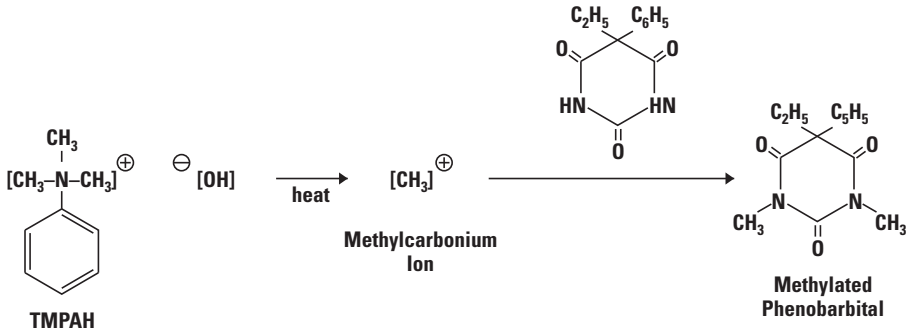



**Figure 1.** Thermo Scientific Pierce MethElute Reagent reaction with phenobarbital.

Thermo Scientific Pierce MethElute Reagent [0.2 M trimethylanilinium hydroxide (TMPAH) in methanol solution] is a powerful methylating reagent for quantitative methylation and detection of barbiturates,[1,2,3] sedatives,[1,3] xanthine bases,[2] phenolic alkaloids[2] and Dilantin[3,4] by gas chromatography.

Our MethElute Reagent gives a single quantitative peak response for each substance. When the reagent is heated with drug-containing extracts, serum or urine, those drugs containing reactive amino, hydroxyl and carboxyl functions will be methylated at the reactive sites.

### Performance Characteristics

**Accuracy.** Comparable or better than the UV/TLC method.[5] When phenobarbital and Dilantin are present, the UV/TLC method cannot be used, as Dilantin interferes with the phenobarbital determination. The GC procedure yields excellent results for this combination of drugs.[5]

**Precision.** The coefficient of variation is 5% or less.

**Sensitivity.** Detects barbiturates down to 0.2 mg/dl.[5]

**References**
1. *Chemical and Engineering News,* April 12, 1971, page 13.
2. Brochmann-Hansen and Oke, T.O. (1969). *J. Pharm. Sci.* **58**, 370-371.
3. Barrett, M.J. (Spring 1971). *The Clinical Chemistry Newsletter* **3(1)**. Published by the Perkin-Elmer Corp., Norwalk, Conn. 06852.
4. Barrett, M.J. (1970). *Clinical Chemistry Application Study No. 33.* Published by the Perkin-Elmer Corp., Norwalk, Conn. 06852.
5. Kananen, G., *et al.* (1972). *J. Chromatogr. Sci.* **10**, 283-287.

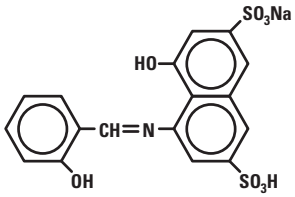
### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 49300 | MethElute Reagent (TMPAH) | 10 ml Hypo-Vial Sample Storage Vial |
| ✖ 49301 | MethElute Reagent (TMPAH) | 12 x 1 ml Hypo-Vial Sample Storage Vials |

✖*Additional hazardous handling charge.*

## Azomethine H Boron Reagent

*Provides rapid boron determination by colorimetry.*




**Azomethine H**
MW 445.41

Thermo Scientific Pierce Azomethine H Boron Reagent provides fast, reliable and sensitive boron determination in soil, plants, composts, manure, water and nutrient solutions.

### Here's how Azomethine H is reported superior to curcumin and other methods:

- Simplicity - fewer steps are involved in the analysis
- Nonacid system; methods carried in water solution
- More accurate results obtained in plant analysis when compared to spectrographic data
- Essentially free from nitrate interference in soil samples with high nitrate levels
- Starting plant material digest or soil extract can be used to determine other important elements

John, *et al.*[1] studied the effects of temperature, time, concentration and composition of the reaction and reagent mixtures. The result is an improved method for the colorimetric determination of boron in soils and plants.[2]

In addition, Gaines and Mitchell showed that the Azomethine H colorimetric boron method gave a certified value of 33 ppm for NBS orchard leaf reference material No. 1571.[3] This is significant because, for the first time, a colorimetric method was given the certified value of an NBS standard. These certified values are otherwise determined with much more sophisticated analytical instrumentation, such as isotope dilution mass spectrometry, nuclear track technique and optical emission spectroscopy.

**References**
1. John, M.K., *et al.* (1975). *Anal. Lett.* **8(8)**, 559-568.
2. Gaines, T.P. and Mitchell, G.A. (1979). *Comm. in Soil Sci. and Plant Anal.* **10(8)**, 1099-1108.
3. Wolf, B. (1971). The determination of boron in soil extracts, plant materials, composts, manures, water and nutrient solutions. *Comm. in Soil Sci. and Plant Anal.* **2(5)**, 363-374.
4. DiLorenzo, A. (1973). Quantitative gas chromatographic analysis of boron trichloride, boron and boron nitride. *J. Chromatogr.* **75**, 207-212.
5. White, C.E., *et al.* (1947). *Anal. Chem.* **19**, 802.

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **40893** | **Azomethine H Boron Reagent** | 25 g |

## Siliconizing Fluids

*An easy way to bond polymer films to surfaces.*

Thermo Scientific Pierce Multifunctional Siliconizing Fluids are specially designed to chemically bind microscopically thin, water-repellent films to glass, quartz, silica and ceramics. The coated surfaces are neutral, hydrophobic and non-oily. In addition, they offer increased resistivity and are not affected by solvents that are not readily hydrolyzed.

### Use our Siliconizing Fluids to treat pipettes, beakers, certain plastics, ceramics, fiber optics and more:

- For clean drainage and elimination of meniscus
- To reduce adsorption of polar compounds, proteins and trace metals onto glass surfaces and reduce leaching of trace metals into solution
- To prevent current tracking and minimize electrical leakage on glass surfaces and ceramics
- To protect delicate samples against the possible reactive effects of -OH sites present on all types of glassware

### More reasons to use our Siliconizing Fluids:

- Easy to apply
- Economical - a little bit goes a long way
- Surfaces can easily be recoated
- Improve surface water-repellency
- Increase surface resistivity

## Pierce Siliconizing Fluid - Water Soluble

*Our water-soluble fluid for siliconizing glass surfaces.*

$$CH_3(CH_2)_{16}CH_2Si(OR)_3$$

Thermo Scientific Pierce Water Soluble Siliconizing Fluid is an easy-to-use silane monomer solution that is supplied as a 20% solid solution in a mixture of diacetone alcohol and tertiary butyl alcohol. The primary silane component is an octadecyltrialkoxysilane that, when mixed with water, is hydrolyzed to a silanol. This silanol condenses with available hydroxyl groups and other silane monomers to form a film on the glass surface.

Our Water Soluble Siliconizing Fluid is especially useful in the biochemical field because of its aqueous phase application to glass and its greater resistance to base hydrolysis.

### Instructions for Use

Our Water Soluble Siliconizing Fluid is applied to a clean glass surface as a dilute aqueous solution. Prepare a 0.1-1.0% solution by weight or volume. [**Note:** Pierce Siliconzing Fluid - Water Soluble contains 20% solids; therefore, one part our Water Soluble Siliconizing Fluid plus 99 parts water (w/w) yields a 0.2% solution - not 1%.] Add the Siliconizing Fluid to water with constant stirring. A clear-to-slightly hazy solution will be obtained. Solutions are not indefinitely stable and will turn cloudy and precipitate after several days and, therefore, solution should be prepared just before use. Solution stability can be optimized, however, by adjusting the aqueous solution pH to 4.5-5.0.

The article to be coated is dipped into the solution, or the surface is flooded with the solution. A thin film will immediately lubricate the glass surface, making it water-repellent. The surface then is air-dried for 24 hours or heated at 100°C for several minutes. Exact drying conditions should be determined before use in commercial process applications.

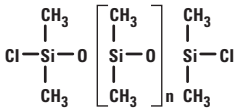
### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 42799 | Pierce Siliconizing Fluid - Water Soluble | 120 ml |

✖*Additional hazardous handling charge.*

## Pierce Siliconizing Fluid - Hydorcarbon Soluble

*A hydrocarbon soluble fluid for siliconizing glass surfaces.*



**Pierce Siliconizing Fluid – Hydrocarbon Soluble**

Thermo Scientific Pierce Hydrocarbon Soluble Siliconizing Fluid is a short chain, clear polymeric silicone fluid consisting primarily of dichlorooctamethyltetrasiloxane. When applied to glass, quartz or similar products, the unhydrolyzed chlorines present on the chain react with surface silanols to form a neutral, hydrophobic and tightly bonded film over the entire surface.

Our Hydrocarbon Soluble Siliconizing Fluid is ideal for use on metals, certain plastics, ceramics and fiber optics.

Our Hydrocarbon Soluble Siliconiziing Fluid is acidic and care should be taken to avoid corrosion of metal that comes into contact with the liquid. The fluid is acidic only during application. After application the surface is neutral.

**Caution:** Material is flammable before film is formed and HCl fumes are generated in the application.

### Instructions for Use

**Wipe-on treatment:** Wearing rubber gloves, wet a cloth with undiluted fluid and rub it on the clean surface until an oily film is formed. Rub with a dry cloth until the surface is clear.

**Solution treatment:** Dilute Pierce Hydrocarbon Soluble Siliconizing Fluid with 1-10% clean dry solvent such as acetone, toluene, carbon tetrachloride, methylene chloride or hexane. Do not use esters or alcohols. Articles can be dipped and air-dried. No heating is required. For a slightly more durable coating, heat articles at 100°C for 30 minutes.

Our Hydrocarbon Soluble Siliconizing Fluid (as supplied) is stable for at least one year. Discard prepared solutions after use.

Jevons, *et al.* reported treating all glassware with a 10% v/v solution of Pierce Siliconizing Fluid - Hydrocarbon Soluble in carbon tetrachloride.[1]


**References**

1. Jevons, S., *et al.* (1971). Biochemistry of blood platelets. Interaction of activated Factor X with platelets. *Biochemistry.* **10(3)**, 428-434.
2. Gershenzon, J., *et al.* (1987). Mechanized techniques for the selective extraction of enzymes from plant epidermal glands. *Anal. Biochem.* **163**, 159-164.

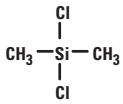

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 42800 | Pierce Siliconizing Fluid-Hydrocarbon Soluble | 120 ml |
| ✖ 42801 | Pierce Siliconizing Fluid-Hydrocarbon Soluble | 480 ml |
| ✖ 42855 | Pierce Siliconizing Fluid-Hydrocarbon Soluble | 5 x 10 ml ampules |

✖*Additional hazardous handling charge.*

## DMDCS

*A more thermally stable way to deactivate polar hydroxyls on glass surfaces.*



**DMDCS**
MW 129.06

Coating with Thermo Scientific Pierce DMDCS is thermally more stable than those achieved with Pierce Water Soluble or Hydrocarbon Soluble Siliconizing Fluids. Use full-strength, or as a 10% solution in an unreactive solvent such as toluene, hexane or methylene chloride. Simply pour reagent through columns or dip article and follow with a methanol rinse. Blow dry with $N_2$ or air.


**Reference**

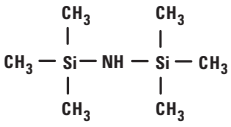
1. Baykut, S., *et al.* (1981). Investigation of physico-chemical behavior of trimethylsilyl derivatives of asymmetric DL-β-hydroxy-β-β'-dialkyl propionic acids on SE 30 liquid phase by gas liquid chromatography. *Chimica Acta Turcia* **9.**


### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 83410 | DMDCS (Dimethyldichlorosilane) | 100 g |

## Monofunctional Silane - HMDS

*Ideal for active surface site deactivation.*



**HMDS**
MW 161.4
bp 125°C
$n_D^{20}$ 1.4071

Thermo Scientific Pierce HMDS is a popular monofunctional silane that many researchers have found useful for deactivating and coating chromatographic supports. Because of its monofunctional nature, this silane can react with only one site on the surface. Polymerization is not possible, eliminating the chances for unbound polymers to float free and elute from the column - avoiding exposure of unreacted silanols beneath the layer. In addition, surface moisture is eliminated because monofunctional reagents dehydrate the surface.

There are several methods for deactivating surfaces with HMDS:

1. Slurrying or dipping the items to be deactivated in a 5-10% solution of the reagent in an unreactive solvent.
2. Vapor phase deactivating by pulling straight vapor into an evacuated container that holds the item to be deactivated.
3. Placing the item and a few milliliters of the reagent in a beaker, then placing a watch glass on top (as in the case of glass wool silanization).

**References**
1. Nawrocki, J. (1985). Modification of silica with mixture, at hexamethylcyclotrisilazane. *Chromatographia* **20(5)**.
2. Owens, N.F., *et al.* (1987). Inhibition of cell adhesion by a synthetic polymer absorbed to glass shown under defined hydrodynamic stress. *J. Cell Sci.* **87**, 667-675.

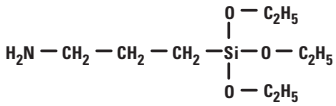
### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 84769 | HMDS (Hexamethyldisilazane) | 100 g |
| ✖ 84770 | HMDS (Hexamethyldisilazane) | 25 g |

✖*Additional hazardous handling charge.*

## 3 -Aminopropyltriethoxysilane

*Useful for preparing amino functional surfaces on glass and silica.*



**3 -Aminopropyltriethoxysilane**
MW 221.37

This monofunctional silane is used for chemically coupling various ligands to glass or silica. The reagent first is coupled to glass or silica. Compounds of interest are coupled to the amino groups directly, or additional chemistries are applied using the amino function before coupling.

### Instructions for Use

1. Dilute reagent to 20% with acetone.
2. Immerse glass.

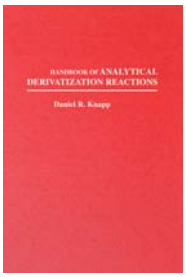
### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 80370 | 3-Aminopropyltriethoxysilane | 100 g |

✖*Additional hazardous handling charge.*

## Handbook of Analytical Derivatization Reaction

*A self-contained methodology reference manual and efficient entry point to the original literature resource book.*



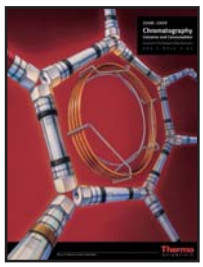

*The Handbook of Analytical Derivatization Reactions* by Daniel R. Knapp is a general collection of analytical derivatization methods for chromatography and mass spectroscopy involving the formation of covalent derivatives before analysis.

Methods contained in this volume are organized according to the type of sample being derivatized. Methods include structural formulas, experimental directions and useful comments. A thorough system of indexing takes you quickly to the "lab ready" methods of interest.

### Ordering Information

| Product # | Description |
|---|---|
| 15012 | **Handbook of Analytical Derivatization Reactions**<br>Knapp, D.R. Ed (1979) Published by John Wiley and Sons, Inc. Hardcover, 741 pages |

## Thermo Scientific Chromatography Columns and Consumables Catalog

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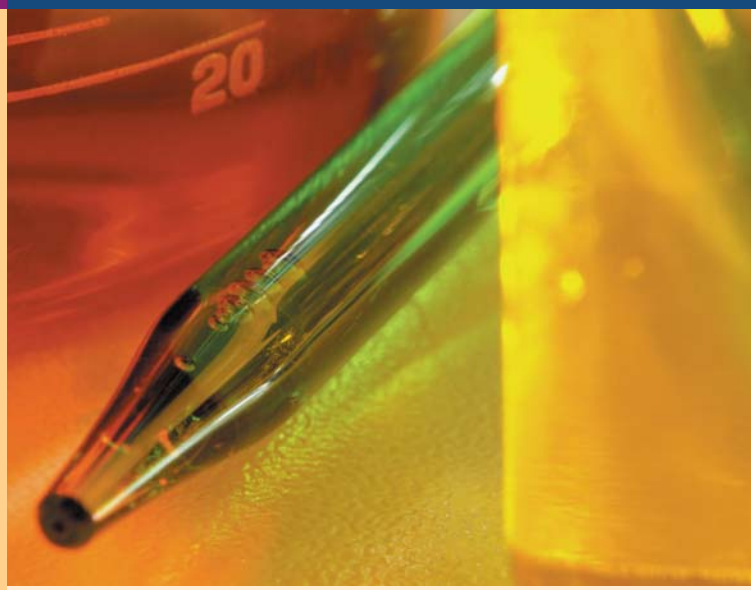
- Thermo Scientific HyperSep SPE products in numerous formats and sorbents including HyperSep Retain Polymeric SPE
- An expanded Thermo Scientific TRACE GC columns line
- A broad range of HPLC and GC accessories
- Hundreds of applications to help identify the most appropriate product for your project
- A convenient technical reference section to help you at the bench, every day

Request your free copy today.

### Ordering Information

| Product # | Description |
|---|---|
| 1601711 | **Chromatography Catalog**<br>340 pages |

# Thermo Scientific Pierce **HPLC Ion Pair Reagents**



## Introduction to HPLC Ion Pair Reagents

*High-purity reagents with the selectivity needed for good separation.*

**In the past, reverse-phase HPLC analysis of highly charged acidic and basic compounds was frustrating and resulted in poor resolution. Important biomolecules such as amino acids, peptides, organic acids, polyamines and catecholamines had to be separated by ion exchange or by suppression techniques.**

Thermo Scientific Pierce Ion Pair Reagents enable you to quickly and efficiently analyze charged compounds using reverse-phase techniques. Our ion pair reagents are simply dissolved in the HPLC solvent system, resulting in the formation of stable chromatographic complexes that can be separated using reverse-phase columns. By using the correct ion pair reagents, you achieve:

- Increased or decreased retention, permitting controlled selectivity
- Resolution of complex ionic mixtures without using ion exchange columns
- Improved peak symmetry

### Reverse-phase ion pair chromatography theories

Two principal theories have been proposed to explain reverse-phase ion pair chromatography. In the first theory, small polar ion pair reagents react with the ionized solute, forming neutral ion pairs. The second theorizes that an active ion exchange surface is produced in which long chain, nonpolar anions and cations are absorbed by the hydrophobic stationary phase.

To optimize chromatographic separations in ion pair elution systems, high-purity reagents of exceptional optical transparency are needed. Pierce Ion Pair Reagents are specially purified for ion pair chromatography and provide the selectivity needed for good separations.


**References**

1. Bennett, H.P.S., *et al.* (1981). *Biochemistry.* **20,** 4530.
2. Starratt, A.N. and Stevens, M.E. (1980). *J. Chromatogr.* **194,** 421.
3. Burgess, A.W., *et al.* (1982). *Proc. Natl. Acad. Sci. U.S.A.* **79,** 5753.
4. Hearn, M.T.W. and Grego, B. (1983). *J. Chromatogr.* **255,** 125.
5. Shoneshofer, M. and Fenner, A. (1981). *J. Chromatogr.* **224,** 472.
6. Fischli, W., *et al.* (1982). *Proc. Natl. Acad. Sci. U.S.A.* **79,** 5435.
7. Hancock, W.S., *et al.* (1979). *J. Chromatogr.* **168,** 377.
8. Hearn, M.T.W., *et al.* (1978). *J. Chromatogr.* **157,** 337.
9. Hearn, M.T.W. and Grego, B. (1983). *J. Chromatogr.* **255,** 125.
10. Hearn, M.T.W. and Grego, B. (1983). *J. Chromatogr.* **266,** 75.
11. Rivier, J. (1978). *J. Liq. Chrom.* **1,** 343.


## Heptafluorobutyric Acid

*An ion pair reagent for the reverse-phase HPLC separation of proteins and peptides.*

### Highlights:

- Clear, colorless liquid
- Typical purity is 99.7% by GC; less than 0.1% water
- Sequencing reagent for classical and automated Edman degradation of peptides and proteins
- Density: 1.645; B.P.: 120°C
- Packaged under nitrogen in amber glass ampules or bottles


**References**

1. Hearn, M.T.W. and Hancock, W.S. (1979). *Trends Biochem. Sci.* **4,** N58-N62.
2. Bennett, H.P.J., *et al.* (1980). *J. Liquid Chromatogr.* **3,** 1353-1366.
3. Bennett, H.P., *et al.* (1981). *Biochemistry* **20,** 4530-4538.


### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 25003 | Heptafluorobutyric Acid, Sequencing Grade | 100 ml |
| ✖ 53104 | Heptafluorobutyric Acid, HPLC Grade | 10 x 1 ml |

✖*Additional hazardous handling charge.*

## Triethylamine (TEA)

*Ideal for HPLC separation and analysis of peptides!*

Triethylamine is an ion-pairing reagent that alters selectivity in reverse-phase HPLC separations. By pairing with peptides, it effectively sharpens peaks, resulting in improved peak resolution. Triethylamine comes in two grades. Our ionate grade is designed for use as an ion-pair reagent in HPLC separations and has a low UV absorbance to provide you with the most sensitive detection across all wavelengths. Sequanal grade is designed to meet the special requirements for peptide sequencing and analysis.

### Highlights:

- > 99.5% triethylamine purity, allowing sensitive peptide detection at low UV wavelengths in reverse-phase HPLC peptide separation systems
- Packaged in amber glass bottles with protective Teflon TFE-lined fluorocarbon caps for reagent integrity

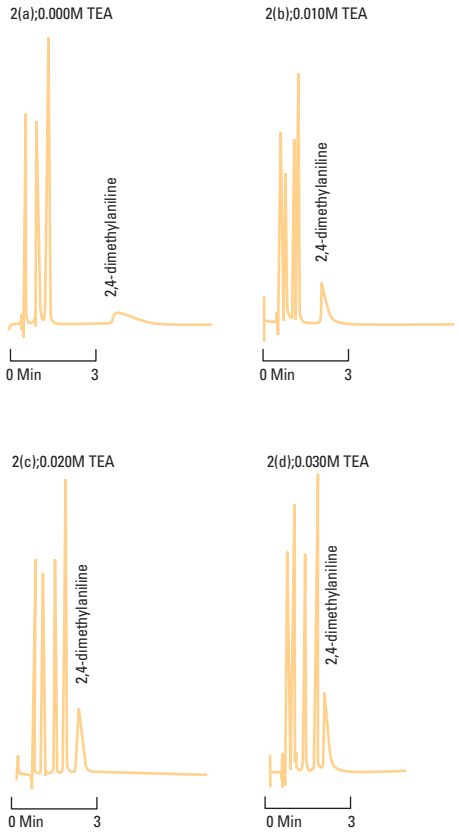



**Figure 1A-1D. Effect of TEA concentration on a mixture of basic antihistamines and 2,4-dimethylaniline* 15 cm x 4.6 mm Zorbax® C8.** Conditions: a) 40% methanol, 0.060 M HSA sodium salt, 0.045 M citric acid; b) 0.150 M citric acid, 0.060 M TEA, pH 7.5 with NaOH; c) 0.150 M citric acid, pH 7.5 with NaOH; isocratic with TEA concentrations modified by varying b/c ratio, 3 min./ml, 50˚C, 254 nm.

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 53101 | Triethylamine (TEA), HPLC Grade | 25 g |
| ✖ 25108 | Triethylamine (TEA), Sequencing Grade | 100 g |

✖*Additional hazardous handling charge.*

## Formic Acid Ampules

*Ideal reagent for LC-MS applications.*

High Purity Thermo Scientific Pierce Formic Acid is sealed in amber glass ampules under a dry nitrogen atmosphere. A pre-measured aliquot of acid greatly simplifies the preparation of liter quantities of mobile phases at the standard 0.1% formic acid concentration. The quality of the formic acid coupled with ampule packaging provides reliability and convenience that adds value to both the chromatographic and MS results.

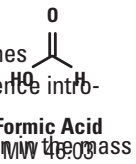
### Formic Acid, Reverse-Phase HPLC and Mass Spectrometry

Formic acid is a component commonly found in reverse-phase mobile phases to provide protons for LC/MS analysis. The presence of a low concentration of formic acid in the mobile phase is also known to improve the peak shapes of the resulting separation. Unlike trifluoroacetic acid (TFA), formic acid is not an ion-pairing agent and it does not suppress MS ionization of polypeptides when used as a mobile-phase component.

### Highlights:

**99% pure formic acid**

- Consistent LC baselines
- No potential interference introduced in LC or MS applications
- No signal suppression in the mass spectrometer



**Formic Acid**
MW 46.03

**High-performance ampule packaging**

- Amber glass, pre-scored, nitrogen-flushed ampules protect formic acid from light and moisture

**Convenient format**

- Ampule packaging simplifies the preparation of gradient and isocratic mobile phases containing 0.1% (v/v) formic acid in water or acetonitrile; the contents of a single vial in a final volume of 1 L of solvent yields a mobile phase of the most common formic acid concentration.

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 28905 | Formic Acid 99+% | 10 x 1 ml ampules |

✖*Additional hazardous handling charge.*

## Trifluoroacetic Acid (TFA)

*1 ml ampules allow you to make a fresh 0.1% TFA solution in seconds!*

Thermo Scientific Pierce Trifluoroacetic Acid (TFA) is the most commonly used ion pairing agent in reverse-phase peptide separations because TFA:

- Sharpens peaks and improves resolution
- Is volatile and easily removed
- Has low absorption within detection wavelengths
- Has a proven history

### Highlights:

**Purity**

- > 99.5% TFA purity and exceptional clarity, allowing sensitive, nondestructive peptide detection at low UV wavelengths in reverse-phase HPLC protein and peptide separation systems

**High-performance packaging**

- Our TFA is packaged under nitrogen in amber glass ampules or bottles with protective Teflon TFE-lined fluorocarbon caps to ensure TFA integrity

**Economical convenience**

- Choose the TFA format that works best for your application. In just a few seconds, 1 ml ampules can be used to prepare 1 liter of fresh 0.1% v/v trifluoroacetic acid solution for the mobile phase in reverse-phase chromatography

### Applications:

- Ion pair reagent for reverse-phase HPLC[1-3]
- Protein/peptide sequencing[4-7]
- Protein/peptide solubilizing agent[4-7]
- Solid-phase peptide synthesis[8]
- Amino acid analysis

### Making 0.1% Solutions of Trifluoroacetic Acid

For complex peptide separations, the key to success can be to vary selectivity. Varying mobile phase composition on the same column can change selectivity enough to resolve peptides that would otherwise overlap. Trifluoroacetic acid is the most frequently used modifier for peptide separations in reverse-phase HPLC. The TFA concentration usually specified is 0.1%. For reproducible separations from run-to-run or from lab-to-lab, it is essential to make TFA concentrations the same.

Trifluoroacetic acid concentration can and should be specified as either "w/v" (weight/volume), or as "v/v" (volume/volume). The w/v specification designates that the TFA is to be weighed and added to a volume of mobile phase (e.g. 0.1% TFA w/v requires one gram of TFA per liter). The v/v specification designates that the TFA is to be measured by volume (e.g. 0.1% TFA v/v requires one ml of TFA per liter).

Because the density of trifluoroacetic acid is 1.53 g/ml the difference between 0.1% TFA (w/v) and 0.1% TFA (v/v) is more than 50%. For the sake of reproducibility, it is essential for authors of a method to specify, and for users of a method to know, whether the TFA concentration is given as "w/v" or "v/v".

**References**

1. Chicz, R.M. and Regnier, F.E. (1990) Methods Enzymol. **182,** 392-421.
2. Zarember, K.A., et al. (2002) Infect. Immun. **70,** 569-576.
3. Lassy, R.A. and Miller, C.G. (2000) J. Bacteriol. **182,** 2536-2543.
4. Smith, B.J. (1997) Protein Sequencing Protocols. Humana Press.
5. Allen, G. (1989) Sequencing of Proteins and Peptides, Second Revised Addition. Elsevier.
6. Backstrom, J.R., et al. (1996) J. Neurosci. **16,** 7910-7919.
7. Hermann, P.M., et al (2000) J. Neurosci. **20,** 6355-6364.
8. Stuart, J.M. and Young, J.D. (1984) Solid Phase Peptide Synthesis, Second Edition. Pierce Chemical Company.

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 28901 | Trifluoroacetic Acid, Sequencing Grade | 500 ml |
| ✖ 28902 | Trifluoroacetic Acid, Sequencing Grade | 10 x 1 g |
| ✖ 28903 | Trifluoroacetic Acid, Sequencing Grade | 100 g |
| ✖ 28904 | Trifluoroacetic Acid, Sequencing Grade | 10 x 1 ml ampules |

✖*Additional hazardous handling charge.*

## HPLC/Spectrophotometric Grade Solvents

*Ultra-pure solvents for HPLC and spectrophotometric applications.*

Thermo Scientific Pierce HPLC Grade Solvents are ultra-pure, distilled in glass, filtered through 0.2 micron Teflon TFE membranes and packed in solvent-rinsed, amber glass bottles. Teflon TFE-lined screw caps seal the bottles for ultimate protection.

Label information includes formula, molecular weight, safety data, flashpoint, first aid procedure, UV absorbance data, GC assay, evaporation residue and water content.

### Physical Properties

#### Acetonitrile, HPLC Grade

- UV Cutoff: 190 nm
- Optical Absorbance: <0.02 at 220 nm
- Refractive Index at 25°C: 1.342

#### Water, HPLC Grade

- UV Cutoff: 190 nm
- Optical Absorbance: <0.005 at 220 nm
- Refractive Index at 25°C: 1.332

#### Dimethylformamide (DMF), Sequencing Grade

- HCON $(CH_3)_2$
- Purity (GC): ≥99%
- MW: 73.09
- Density: 0.944
- B.P. 153°C
- Water: <0.1%

#### Dimethylsulfoxide (DMSO), Sequencing Grade

- $C_2H_60S$
- Purity (GC): >99.5%
- MW: 78.13
- Density: 1.101
- Water: ≤0.2%

#### Pyridine

- $C_{5H}{}^{5N}$
- Purity (GC): ≥99%
- MW: 79.10
- Density: 0.978
- B.P.: 115°C

### Ordering Information

| | Product # | Description | Pkg. Size |
|---|---|---|---|
| ✖ | 51101 | Acetonitrile | 1 L |
| | 51140 | Water | 1 L |
| ✖ | 20673 | Dimethylformamide (DMF) | 50 ml |
| | 20688 | Dimethylsulfoxide (DMSO), Sequencing Grade | 950 ml |
| | 25104 | Pyridine | 100 g |

✖*Additional hazardous handling charge.*
*For GC Grade Solvents, see page 18.*

## Peptide Retention Standard for Reverse-Phase HPLC

*Increases the efficiency of peptide elution profile predictions.*

A simple, quantitative method for predicting peptide retention times was developed by Guo, *et. al.* [1-3] Retention times are predicted by totaling the values that represent the contribution in minutes of each amino acid residue and the peptide terminal groups.

Retention time is dependent upon the molecular weight of the peptide. The effect on retention is relatively unimportant with a small peptide, but it increases with the size of the molecule. The accuracy of predicting peptide retention time significantly decreases beyond 20 residues.

To ensure accuracy, a peptide standard is used to correct for instrument variation, column aging, n-alkyl chain length variation and ligand density.

By using Thermo Scientific Pierce Peptide Retention Standard, you can:

- Determine the relative order of peptide elution of a complex mixture
- Increase the efficiency of predicting peptide elution profiles
- Save time in peptide purification
- Simplify identification of specific peptides in a complex mixture
- Predict the HPLC retention time for peptides of known amino acid composition on reverse-phase HPLC columns
- Monitor column performance - efficiency, selectivity and resolution during column aging
- Compare reverse-phase columns from different manufacturers
- Evaluate reverse-phase supports of varying n-alkyl chain lengths and ligand densities

**References**

1. Guo, D., *et al.* (1985). *Proceedings of the Ninth American Peptide Symposium,* Published by Pierce, Rockford, Illinois, page 23.
2. Guo, D., *et al.* (1986). *J. Chromatogr.* **359,** 499-517.
3. Guo, D., *et al.* (1986). *J. Chromatogr.* **359,** 519-532.
4. Mant, C.T. and Hodges, R.S. (1986). *L.C. Magazine Liq. Chrom. and HPLC* **4(3),** 250.
5. Guo, D., *et al.* (1987). *J. Chromatogr.* **386,** 205-222.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 31700 | Peptide Retention Standard, S1-S5<br>Contains: 5 C-terminal amide decapeptides, 4 of which are $N^{\alpha}$-acetylated with the sequence variation as follows: AC-Arg-Gly-X-X-Gly-Leu-Gly-Leu-Gly-Lys-Amide; $Gly^3$-$Gly^4$-$Ala^3$-$Gly^4$, $Val^3$-$Gly^4$ and $Val^3$-$Val^4$. The fifth peptide, $Ala^3$-$Gly^4$, contains a free N$\alpha$-amino group. This mixture will provide 100-200 injections at 0.1 AUFS at 210 nm. | 1 vial |

## Thermo Scientific Pierce Derivatization Reagents for HPLC

*Designed to provide selectivity and improve sensitivity.*

The lack of a universal HPLC detector that provides high sensitivity (as well as some degree of selectivity) established the need for suitable derivatization procedures. Derivatization is the chemical modification of an existing compound, producing a new compound that has properties more suitable for a specific analytical procedure. It is an analytical tool that can be used to provide both selectivity and improved sensitivity.

There are several requirements for derivatization protocol:

1. At least one acidic, polar functional group must be available for reaction on the parent compound.
2. A single derivative should be formed per parent compound.
3. The reaction should be reproducible under the given time and reaction conditions.
4. The reaction should proceed quickly and easily under mild conditions.
5. The reaction byproducts (if any) should not interfere with the chromatography, or with detection of the sample.

Pre- and post-chromatographic techniques are both used in HPLC derivatization. In addition, off-line and on-line reactions have been employed with both techniques. Pre-chromatographic (or pre-column techniques) offer more than greater selectivity and sensitivity in detection. Pre-column techniques can be used to enhance stability, improve resolution, improve peak symmetry and increase or decrease retention of solutes. FDAA (Marfey's Reagent) allows separation and quantification of optical isomers of amino acids (Figure 1). Post-chromatographic (or post-column) techniques are used primarily to provide selectivity and improve sensitivity.

We offer a variety of HPLC detection reagents for pre- and post-chromatographic techniques. All compounds and formulations are purified for chromatography, minimizing artifact formation.

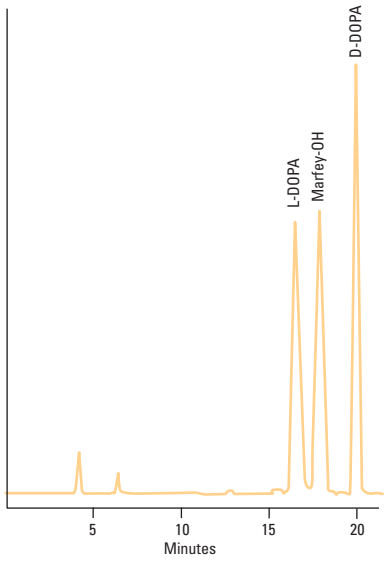



**Figure 1. Separation of D- and L-DOPA on Spheri-5™ Sorbent, RP-18. 10 cm x 4.6 mm.** Conditions: A) 0.05 M triethylamine phosphate, pH 3.0; B) acetonitrile. Linear gradient: 10 to 40% B in 45 minutes, 2.0 ml/minute, 25°C, 340 nm.

## FDAA, Marfey's Reagent

*Makes it quick and easy for you to separate and quantitate optical isomers of amino acids by reverse-phase HPLC.*

Optical isomers of amino acids can be simply and conveniently derivatized with Thermo Scientific Pierce FDAA, Marfey's Reagent (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide) - and preparation is complete in just 90 minutes.

With Marfey's Reagent, the amino acid derivatives can easily be separated and quantitated by reverse-phase HPLC. Derivatives have an absorption coefficient of ~3 x $10^4$ and can be detected by UV at 340 nm with picomole sensitivity.

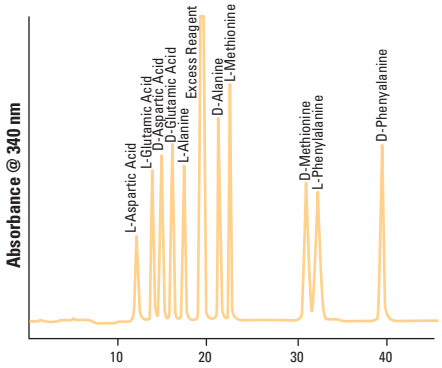
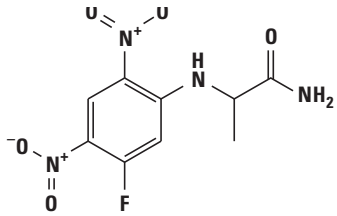



**Figure 1. Separation of D- and L-amino acids on Spheri-5 Sorbent, RP-18. 10 cm x 4.6 mm.** Conditions: A) 0.05 M triethylamine phosphate, pH 3.0; B) acetonitrile. Linear gradient: 10 to 40% B in 45 minutes, 2.0 ml/minute, 25˚C, 340 nm.



**FDAA**
**(Marfey's Reagent)**
MW 272.19

### PROTOCOL

**Preparation of FDAA Derivatives**

1. Place 100 µl (5 µmol) sample in a 1.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Add 200 µl of a 1% (w/v) solution of Pierce FDAA in acetone. Add 40 µl of 1.0 M sodium bicarbonate. µmol FDAA: µmol amino acid should be 1.5:1.0.
3. Heat at 40˚C for 1 hour. Remove and cool.
4. Add 20 µl 2 M HCl. Allow sample to degas.
5. Analyze. Conditions:
   Spheri-5 Sorbent, RP-18, 10 cm x 4.6 cm
   UV at 340 nm
   A: 0.05 M TEA phosphate, pH 3.0
   B: CH3CN
   Linear gradient: 10% B to 40% in 45 minutes
   Flow: 2.0 ml/minute at 25˚C

**Reference**

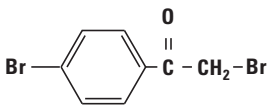
1. Marfey, P. (1984). *Carlsberg Res. Comm.* **49,** 591-596.

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 48895 | FDAA, Marfey's Reagent (1-fluoro-2,4-dinitrophenyl-5-L-alanine-amide) | 50 mg |

## *p*-Bromophenacylate Reagent

*Procedure gives quantitative yields with few or no side reactions.*




***p*-Bromophenacylate**
MW 277.94

Durst, *et al.* have described a novel preparation of various phenacyl esters and their use as UV visualizing agents in the 1-10 ng range. This procedure gives quantitative yields with few or no side reactions. Phenacyl esters have been used to separate many saturated and unsaturated fatty acids,[2,3] including prostaglandins.[4]

Phenacyl esters have some significant advantages over previously reported methods, including:

- Pre-mixing of phenacylbromide and crown ether is not necessary
- Derivatization is both rapid and quantitative, with yields of more than 95% in 15-20 minutes at 80˚C
- Excess reactants do not interfere
- Large excess of alkylating reagent is not necessary
- Small amounts of water or alcohol do not interfere
- If isolation is desired, products usually are crystalline

### PROTOCOL

#### Preparation of Phenacyl Esters

**Pierce *p*-Bromophenacylate Reagent** (0.1 µmol/ml *p*-Bromophenacylbromide, 0.005 µmol/ml crown ether in acetonitrile)

1. Dissolve ~10 mg acid in MeOH in a 5.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial fitted with Thermo Scientific Reacti-Vial Magnetic Stirrer. Neutralize to the phenolphthalein endpoint with KOH/MeOH.*
2. Evaporate the MeOH with $N_2$.
3. Add 1.0 ml Phenacylate Reagent and 2.0 ml dry $CH_3CN$.
4. Heat at 80˚C with stirring for 30 minutes.
5. Remove and cool.
6. Analyze. Conditions:
   Spheri-5, RP-18
   UV at 250 nm
   A: $CH_3CN$
   B: deionized $H_20$
   Linear gradient: 80% A to 100% A
   Flow: 2.0 ml/minute

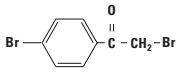
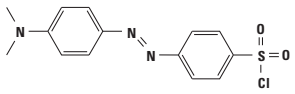
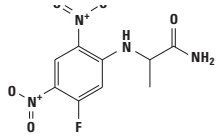
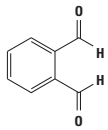
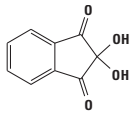
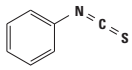
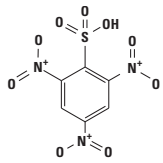
** If the formation of potassium salts is undesirable, neutralize by adding $KHCO_3$ at five times the total acid instead of using KOH.*

**References**
1. Durst, H.D., *et al.* (1975). *Anal. Chem.* **47,** 1797.
2. Borch, R.F., *et al.* (1975). *Anal. Chem.* **47,** 2437.
3. Grushka, E., *et al.* (1975). *J. Chromatogr.* **112,** 673.
4. Fitzpatrick, F.A. (1976). *Anal. Chem.* **48,** 499.
5. Nagels, L., *et al.* (1980). *J. Chromatogr.* **190,** 411.
6. Ahmed, M.S., *et al.* (1980). *J. Chromatogr.* **192,** 387.
7. Pierce Technical Bulletin: Preparation of phenacyl and *p*-Bromophenacyl derivatives for HPLC.
8. Patience, R.L. (1982). *J. Chromatogr.* **249,** 183-186.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **48891** | **p-Bromophenacylate Reagent**<br>0.1 mmol/ml p-Bromophenacylbromide,<br>0.005 mmol/ml crown ether in acetonitrile | 10 ml Hypo-Vial<br>Sample<br>Storage Vial |

## Thermo Scientific Pierce Detection Reagents for HPLC.

| Functional Group | Description | Detection* | Page | Comments |
|---|---|---|---|---|
| **Carboxylic Acid**  | ***p*-Bromophenacylate**  | UV | 38 | Formulation of 1.0 mmol/ml *p*-bromophenacyl bromide and 0.005 mmol/ml crown ether in acetonitrile; pre-column; nanomole detection levels: $\lambda_{max}$ = 260 nm[1 7] |
| **Primary Amine**  | **Dabsyl Chloride**  | Vis | 52 | 4-N, N-dimethylaminoazobenzene-4'-sulfonyl chloride (dabsyl chloride); pre-column; nanomole detection levels: $\lambda_{max}$ = 436 nm[8 14] |
| | **FDAA, Marfey's Reagent**  | UV | 37, 52 | 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA); pre-column; nanomole detection levels: $\lambda_{max}$ = 340 nm. For chiral separations of amino acids.[15 28 29] |
| | **Fluoraldehyde**  | EC, F | 47, 48, 51 | Formulation of 0.8 mg OPA/ml in 1 M potassium borate buffer, pH 10.4 containing Brij-35 and β-mercaptoethanol; pre- or post-column; picomole detection levels with EC and F; GC + 0.5 1.0 V; $\lambda_{ex}$ = 360 cm, $\lambda_{em}$ = 455 nm[16 21] |
| | **Ninhydrin**  | Vis | 50 | Post-column; nanomole detection levels: $\lambda_{max}$ = 570 nm[22] |
| | **PITC**  | UV | 51 | Phenylisothiocyanate (PITC); pre-column; picomole detection levels: $\lambda_{max}$ = 254 nm[23 24] |
| | **TNBSA**  **TNBSA** MW 293.17 | EC, UV | 48 | 2,4,6-Trinitrobenzene-sulfonic acid (TNBSA); pre- or post-column; nanomole detection levels with EC and UV, GC - 0.85V; $\lambda_{max}$ = 250 nm[25 26] |
| **Secondary Amine**  | **Ninhydrin** (see structure above) | Vis | 50 | Post-column; nanomole detection levels: $\lambda_{max}$ = 440 nm[22] |
| | **PITC** (see structure above) | UV | 51 | Phenylisothiocyanate (PITC); pre-column; picomole detection levels: $\lambda_{max}$ = 254 nm[23 24] |

**EC = electrochemical; F = fluorescence; UV = ultraviolet; Vis = visible.*

## Detection Reagents

**References**

1. Durst, H.D., *et al.* (1975). *Anal. Chem.* **47,** 1797.
2. Borch, R.F., *et al.* (1975). *Anal. Chem.* **47,** 2437.
3. Grushka, E. *et al.* (1975). *J. Chromatogr.* **112,** 673.
4. Fitzpatrick, F.A. (1976). *Anal. Chem.* **48,** 499.
5. Nagels, L., *et al.* (1980). *J. Chromatogr.* **190,** 411.
6. Ahmed, M.S., *et al.* (1980). *J. Chromatogr.* **192,** 387.
7. Pierce Technical Bulletin: Preparation of Phenacyl and *p*-Bromophenacyl Derivatives for HPLC.
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9. Chang, J.Y., *et al.*. (1981). *Biochem. J.* **199,** 547-555.
10. Chang, J.Y., *et al.* (1982). *Biochem. J.* **203,** 803-806.
11. Vendrell, J., *et al.* (1986). *J. Chromatogr.* **358,** 401-413.
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17. Fiedler, H.P., *et al.* (1986). *J. Chromatogr.* **353,** 201-206.
18. Fried, V.A., *et al.* (1985). *Anal. Biochem.* **146,** 271-276.
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20. Seaver, S.S., *et al.* (Sept./Oct. 1984). *Biotechniques* 254-260.
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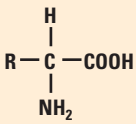
22. Stein, W.H. and Moore, S. (1949). *Cold Spring Harbor Symp. Quant. Bio.* **14,** 179.
23. Heinrickson, R.L. and Meredith, S.C. (1984). *Anal. Biochem.* 137, 65-74.
24. Scholze, H. (1985). *J. Chromatogr.* **350,** 453-460.
25. Caudill, W.L., *et al.* (1982). *J. Chromatogr.* 227, 331.
26. Caudill, W.L., *et al.* (1982). *Bioanalytical System's Current Separations* **4(4),** 59.
27. Andrews, J.L., *et al.* (1982). *Arch. Biochem. Biophys.* **214,** 386-396.
28. Aberhart, D.J., *et al.* (1985). *Anal. Biochem.* **151,** 88-91.
29. Szokan, G., *et al.* (1988). *J. Chromatogr.* **444,** 115-122.

# Introduction to Amino Acid Analysis

## The History

**The development of amino acid analysis began in 1820 when Braconnot isolated glycine from a hydrolyzate of gelatin.[1] Later, in 1848, the Dutch chemist Mulder showed that glycine contains nitrogen, a major component of amino acids.[2] It was not until 1883, however, that Kjeldahl introduced a method that accurately determined the amount of nitrogen in a protein/amino acid sample.[3]**

By 1910, most of the amino acids had been isolated and their structures discovered. As the number of known amino acids accumulated, it became possible to group them on the basis of common chemical features. At that time, it was discovered that all amino acids have the same general formula and differ only by the chemical structure of the side chains.




From 1910-1940, amino acid research was characterized by the work of quantitative analysts, as opposed to the organic chemists of the 1800s. Amino acid analyses conducted during the 1800s and early 1900s were laborious, often extending over weeks and months. While the amino acid content of a number of proteins was discovered, exact information was not always obtainable using the equipment available during that time period.[4]

The introduction of chromatography opened new doors for amino acid analysis. The first breakthrough came when Martin and Synge introduced partition chromatography, which separates the acetyl derivatives of certain amino acids.[5] In this method, an equilibrium is established between two liquid phases. Silica gel is mixed with a solution of water and an indicator. The resulting slurry is packed into a column, forming the stationary phase. Next, the acetyl amino acids are dissolved in solvent, forming the mobile phase. The acids then are placed in the same column. The acetyl amino acids flow through the column at different rates. Separation is made visible by the bands of color change in the indicator.

While this system successfully separated mono-amino and mono-carboxylic acids, it was impractical for other types of amino acids.

Later, Martin and his associates used filter paper as an alternative to silica gel, developing a paper chromatography method that is still in use today. The amino acids were dissolved in butanol and allowed to seep onto the filter paper for a set amount of time. The paper then was dried and sprayed with a dilute solution of ninhydrin (2,2-dihydroxy-1,3-indandione) in butanol. The colored spots were measured and compared with the set values for those experimental conditions.

The separations achieved with paper chromatography were only semi-quantitative. Column chromatography, on the other hand, had potential for quantitation, but the separations were imperfect. The introduction of ion exchange chromatography solved these problems, allowing column separation of amino acids without any prior derivatization.[6,7] Initially used to remove carbohydrate contamination from starch columns, ion exchange resins were quickly found to have great potential for separating amino acids. While many types of polymeric exchange resins were tested, polysulfonic resins (such as Dowex 50) provided the best separations.[8]

Modifications to these procedures have improved amino acid separations. Resin characteristics, column size, column temperature, buffer pH and ionic strength all have been modified to improve resolution of amino acid mixtures and achieve specific separations. Also, quantitation was greatly improved by the use of post-column reactions with ninhydrin. At one time ninhydrin was the most widely used detection system; however, more sensitive indicators, such as *o*-phthalaldehyde were developed to increase analytical sensitivity.

## Developments in Amino Acid Analysis

Improvements in amino acid analysis by ion exchange chromatography have involved the analytical system, as well as the instrumentation. Systems have been developed (by varying buffer pH or ionic strength) that work to displace the amino acids into discrete bands. The buffer systems are compatible with single- or two-column analysis of amino acids found in protein hydrolyzates or physiological fluids. Buffer systems are determined by the counter ion used (sodium or lithium) and by the method of buffer changes introduced to the resin (step changes or gradient elution).[9-15] The most commonly used buffering component, citrate, is suitable for solutions below pH 7.[16] Buffers are prepared either with citric acid or an alkali salt, and citrate concentrations of 0.05 to 0.06 7 M are common.

Unfortunately, for high-sensitivity work, citric acid is a significant contributor to amino acid contamination. Therefore, to achieve consistent analyses, it is essential to use high-purity reagents for buffer preparation.

Alternatives to ion exchange are available for the separation of amino acids. Because amino acid analysis is one of the basic protein chemistry tools available, more rapid and sensitive methods for separation and quantitation are desirable.[17] Several pre-column derivatization methods using reverse-phase HPLC have been developed.

Two of the most widely used of these methods involve the formation of dansyl[18-19] or *o*-phthalaldehyde (OPA)[20-24] derivatives of amino acids prior to HPLC analysis. Both methods offer greater sensitivity and shorter analysis time than post-column derivatization techniques. Other methods include the quantitative derivatization of amino acids with phenylisothiocyanate (PITC) and the separation and quantitation of the resulting phenylthiocarbonyl derivatives via HPLC. These derivatives are stable enough to eliminate in-line derivatization.

# Sample Preparation and Hydrolysis

The extraction and purification of proteins play an important role in determining amino acid content. These methods are based on one or more of their physical characteristics (e.g., solubility, molecular size, charge, polarity and specific covalent or noncovalent interactions). The techniques commonly used to separate proteins and peptides include:

- Reverse-phase HPLC
- Polyacrylamide gel electrophoresis
- Gel filtration
- Ion exchange chromatography
- Affinity chromatography

Table 1 provides a more detailed list of methods for fractionating peptide mixtures.[25]

**Table 1. Methods for the fractionation of peptide mixtures.**

| Technique | Properties of Peptide Molecules Exploited |
|---|---|
| Centrifugation | Solubility |
| Size exclusion chromatography | Size |
| Ion exchange chromatography | Charge, with some influence of polarity |
| Paper electrophoresis | Charge and size |
| Paper chromatography | Polarity |
| Thin layer electrophoresis | Charge and size |
| Thin layer chromatography | Polarity |
| Polyacrylamide gel electrophoresis | Charge and size |
| High-performance liquid chromatography (HPLC) | Polarity |
| Gas chromatography | Volatility of derivatives |
| Counter-current extraction | Polarity; sometimes specific interactions |
| Affinity chromatography | Specific interactions |
| Covalent chromatography or irreversible binding | Disulfide bond formation; reactivity of homoserine lactone |

## Hydrolysis

Most protein samples require some form of chemical treatment before their component amino acids are suitable for analysis. Protein and peptide samples must be hydrolyzed to free amino acids from peptide linkages. Acids (usually HCl) are the most widely used agents for hydrolyzing proteins.

A simplified hydrolysis procedure involves refluxing the protein with excess HCl, then removing the excess acid in vacuum.[26] The lyophilized protein then is suspended in constant boiling 6 N HCl and introduced into the hydrolysis tube. The sample is frozen by immersing the tube in dry ice and acetone. Before sealing, the tube is evacuated to avoid formation of cysteic acid, methionine sulfoxide and chlorotyrosine.[27] This procedure minimizes decomposition of reduced S-carboxymethylcysteine and analyzes S-carboxymethylated proteins. Hydrolysis is conducted at 110°C (with the temperature accurately controlled) for 20-70 hours by Moore and Stein's method.[28] After hydrolysis, residual HCl is removed in a rotary evaporator. The residue is dissolved in water and brought to the appropriate pH for addition to the analyzer column.[28]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## Vacuum Hydrolysis Tubes

*Completely reusable and compatible with Reacti-Therm Modules.*

Thermo Scientific Reacti-Therm Systems deliver uniform dry heat with unmatched convenience and versatility, making them an ideal choice for hydrolosis reactions.

### Applications:

- Hydrolysis
- Sample concentration
- Lyophilization
- Hydrazinolysis

### PROTOCOL

**A. Preparation**

1. Unscrew the Teflon plug from the Thermo Scientific Vacuum Hydrolysis tube, 5ml (Product # 29560).
2. Using a small diameter glass pipette (disposable Pasteur) or syringe, introduce the sample into the bottom reservoir of the tube. Avoid leaving any residue of the sample in the upper portion of the tube, particularly in the area of the threads, and at the top of the reservoir where the Teflon plug makes a seal.
3. Add the specially purified Pierce Constant Boiling Hydrochloric Acid (Product # 24308) up to the desired reaction level.

**NOTE:** For best results, the total volume of sample and hydrochloric acid should not exceed 1/3 of the stated reservoir volume. This will help prevent loss during the application of vacuum, when bumping and foaming may occur. To prevent excessive bumping and foaming during the vacuum process, the sample can be frozen prior to the application of vacuum.

**B. Application of Vacuum**

1. Insert the inert Teflon plug and screw it down just enough to leave a small passageway between the plug and the glass at the constricture point.
2. Secure the tube and affix the vacuum source to the side arm. It is helpful to use vacuum tubing to connect the hydrolysis tubes to a three-way stopcock. The stopcock should be connected to a vacuum source and a supply of purified nitrogen or argon. This will allow you to alternate between vacuum and an inert gas, which ensures the complete removal of oxygen from the sample. Additionally, this set-up will facilitate the controlled release of the vacuum after the hydrolysis is complete.

**C. Hydrolysis**

1. When the desired vacuum is reached, seal the unit by slowly screwing the plug down until it is flush with the glass surface at the constricture. Do not overtighten! Applying too much pressure to the hydrolysis tube is the most common cause of damage to the tube or Teflon plug. Our Vacuum Hydrolysis Tubes are designed to withstand temperatures up to 200°C for 48 hours.

**NOTE:** At 150°C, specially purified hydrochloric acid allows rapid (6-hour) hydrolysis of proteins. (Standard protein hydrolysis conditions are 105-110°C for 16-24 hours.)

2. After the tubes have cooled, release the Teflon plug just enough to create a small passageway between the plug and the glass constricture. The three-way stopcock set-up, described earlier, facilitates this procedure and allows you to break the vacuum without allowing air/oxygen into your sample.

**D. Sample Concentration**

1. After securely fastening the tube, your sample can be concentrated (via vacuum) with or without heating the tube. Alternatively, the hydrolysis reagent may be removed by lyophilization, or via a stream of nitrogen/argon directed to the surface of the sample.
2. At the appropriate point in your procedure, use a small diameter pipette to remove your sample. The hydrolyzed sample can easily be redissolved in the sodium citrate sample dilution buffer.
3. Completely remove the Teflon plug and use a small pipette to add the appropriate quantity of buffer to the dried sample.
4. Allow the sample to dissolve and remove it with a pipette.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 29550 | **Vacuum Hydrolysis Tube**<br>8 mm x 60 mm, 1 ml volume | Each |
| 29560 | **Vacuum Hydrolysis Tube**<br>10 mm x 100 mm, 5 ml volume | Each |
| 29564 | **Vacuum Hydrolysis Tube**<br>19 mm x 100 mm, 20 ml volume | Each |

## Reacti-Block Aluminum Blocks For Vacuum Reaction Tubes

*For use with Reacti-Therm Heating/Stirring Modules.*

Thermo Scientific ReactiTherm Systems deliver uniform dry heat with unmatched convenience and versatility, making them an ideal choice for hydrolysis reactions.

### Ordering Information

| Product # | Description |
|---|---|
| 18806 | **Reacti-Block F**<br>Holds 8 x 5 ml Vacuum Hydrolysis Tubes; 8 holes/10 mm dia. x 64 mm deep |
| 18807 | **Reacti-Block G**<br>Holds 4 x 20 ml Vacuum Hydrolysis Tubes; 4 holes/19 mm dia. x 64 mm deep |
| 18819 | **Reacti-Block V-1**<br>Holds 17 Microcentrifuge Test Tubes; 17 holes/11 mm dia. x 44 mm deep |

## Constant Boiling (6N) Hydrochloric Acid

*For total protein hydrolysis.*

Thermo Scientific Pierce Hydrochloric Acid is purified and packaged to ensure a ninhydrin negative blank on hydrolysis. Convenient, pre-scored ampule packaging of the ready-to-use HCl maintains reagent integrity. This virtually eliminates exposure to laboratory atmospheres, fingerprints and other contaminants resulting from pipetting from bulk bottles.

Eveleigh and Winter give an excellent description of the total protein hydrolysis technique using Constant Boiling Hydrochloric Acid.[1] Standard protein hydrolysis conditions are 105-110°C for 16-24 hours. At 150°C, this reagent can hydrolyze peptides in 6 hours.

### Highlights:

- Hydrolyzes peptides in 6 hours at 150°C
- Specially purified to give ninhydrin-negative blank on hydrolysis
- Packaged in ampules to eliminate contamination and ensure product integrity

**References**

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### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖24308 | Hydrochloric Acid [Constant boiling, Hydrochloric Acid 6N Sequencing Grade] | 10 x 1 ml ampules |

✖*Additional hazardous handling charge.*

## Amino Acid Standard H

*Our high-purity amino acid calibration standard for protein hydrolyzates.*

The high-purity amino acids of Thermo Scientific Pierce Amino Acid Standard H are ideal for calibrating amino acid analyzers. To permit standardization of microbiological and other assays, we have used the L-form configuration. The molar concentration of each standard is verified by conventional amino acid analysis methods.

With the exception of cystine, each amino acid is supplied at a concentration of 2.5 µmoles/ml in 0.1 N HCl. The following amino acids are included with our Amino Acid Standard H:

- L-Alanine
- Ammonia [$(NH_4)_2$ $SO_4$]
- L-Arginine
- L-Aspartic Acid
- L-Cystine
- L-Glutamic Acid
- Glycine
- L-Histidine
- L-Isoleucine
- L-Leucine
- L-Lysine•HCl
- L-Methionine
- L-Phenylalanine
- L-Proline
- L-Serine
- L-Threonine
- L-Tyrosine
- L-Valine

### Instructions for Use

Thaw Standard H and shake well. Dilute appropriately with suitable buffers to a concentration compatible with the full-scale sensitivity of your amino acid analyzer.

### Storage

When kept frozen, an unopened vial has an indefinite storage life. Once the seal is broken, the reagent has a maximum storage life of six months. Store Pierce Amino Acid Standard H frozen between uses.

### Ordering Information

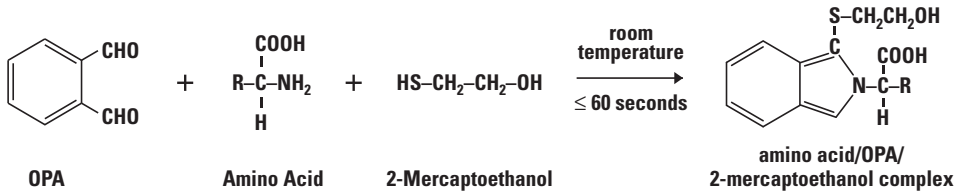
| Product # | Description | Pkg. Size |
|---|---|---|
| 20088 | Amino Acid Standard H | 10 x 1 ml ampules |

## *o*-Phthalaldehyde Detection of Amino Acids and Primary Amines

Ion exchange chromatography of amino acids, followed by post-column reaction with the highly sensitive *o*-phthalaldehyde (OPA) fluorophoric reagent, allows measurement of amino acids at the sub-nanomole level.[1-8]

High-sensitivity detection is achieved by post-column reaction of amino acids with OPA/2-mercaptoethanol in a potassium borate buffer. The amino acid/OPA/2-mercaptoethanol reaction complex has an excitation wavelength of 360 nm and fluoresces at 455 nm. This reaction is complete in less than one minute at room temperature.[2]

The OPA technique has been reported to be 10 times more sensitive than the ninhydrin reaction. Detection of secondary amines, such as proline and hydroxyproline, is not possible unless they are first oxidized with a dilute solution of sodium hypochlorite. Under alkaline conditions, sodium hypochlorite converts the secondary amines (imino acids) to primary amines, generating fluorescence after reacting with OPA.[10] Recent improvements,[3-4] along with the adaptation of hypochlorite oxidation for proline analysis,[8,10-12] makes the OPA/2-mercaptoethanol technique suitable for routine analysis.




**Reaction Scheme 1.** *o*-Phthalaldehyde reaction with an amino acid in presence of 2-mercaptoethanol. Reaction occurs quickly at room temperature to form an amino acid/OPA/2-mercaptoethanol fluorescent complex.

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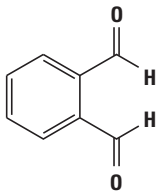
## Fluoraldehyde *o*-Phthalaldehyde (OPA) Reagent Solution

*Great for pre- or post-column fluorescent detection of amines.*[1,2,5]



**Thermo Scientific Pierce Fluoraldehyde Reagent Solution (*o*-Phthalaldehyde)**
MW 134.13
λex = 340 nm
λem = 455 nm

Thermo Scientific Pierce Fluoraldehyde Reagent Solution contains a stabilized, highly purified preparation of *o*-phthaladehyde, Brij®-35 Detergent and mercaptoethanol in a specially formulated borate buffer. It is a highly sensitive, ready-to-use reagent solution that exhibits excellent linear response (Figure 1) and offers outstanding shelf life (Figure 2). In addition, when compared to other *o*-phthalaldehyde detection reagents, our solution exhibits decreased background over time and a high signal-to-noise ratio.

*See page 51 for pre-column protocol using Pierce Fluoraldehyde OPA Reagent Solution*

**References**
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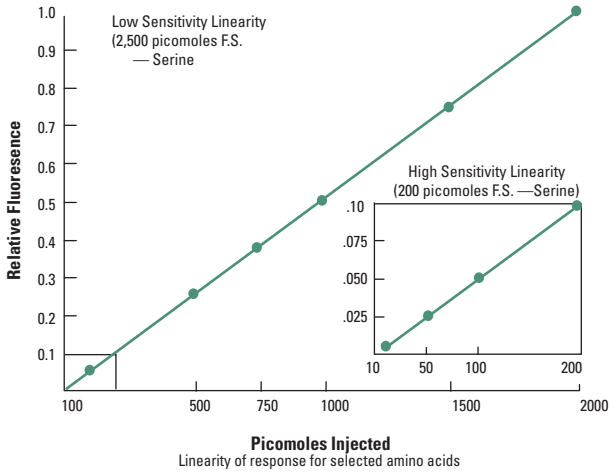
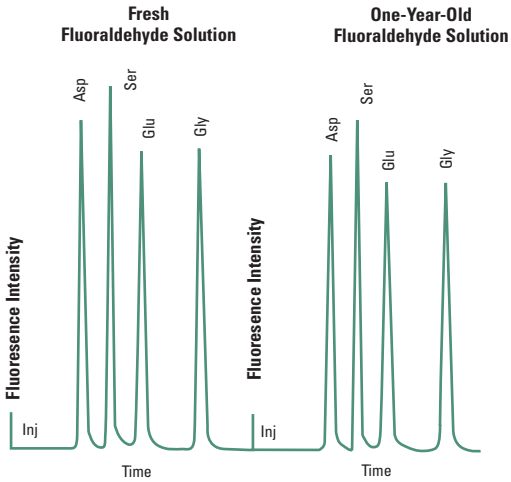


Linearity of response for selected amino acids

**Figure 1. Excellent linear response.** Thermo Scientific Pierce Fluoraldehyde Reagent Solution shows excellent linear response, whether in the 2,500 or 200 picomole range.



**500 picomole analyses of selected amino acids**

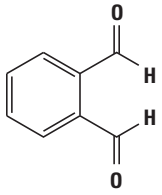
**Figure 2. Outstanding shelf life.** Comparison of fluorescence response of selected amino acids after reaction with recently prepared and one-year-old Thermo Scientific Pierce Fluoraldehyde Reagent Solutions.

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **26025** | **Fluoraldehyde Reagent Solution** (0.8 mg/ml *o*-phthalaldehyde) | 945 ml |

## Fluoraldehyde Crystals

*An easy-to-use, yet economical way to detect amino acids in pre-[1] and post-column[2,3] chromatographic effluents.*



**Thermo Scientific Pierce Fluoraldehyde Crystals (*o*-Phthalaldehyde)**
MW 134.13

Thermo Scientific Pierce Fluoraldehyde Crystals are stable in aqueous solution, making them a highly sensitive, yet economical and easy-to-use purified grade of crystalline *o*-phthaladehyde. While no heating is required with Fluoraldehyde Crystals, they allow rapid analysis and exhibit low background.

A common procedure involves dissolving our Fluoraldehyde Crystals in a pH 10.4 potassium borate buffer solution for a final concentration of 0.8 mg/ml.

### Applications

- Post-column cysteine and cystine derivatization[2]
- Automated pre-column derivatization of plasma amino acids[3]
- Pre-column derivatization of free physiological amino acids in tissues and biological fluids[4]
- Derivatization of amino acids from biopsy specimens. Comparison of OPA reagents using β-mercaptoethanol, ethane thiol or mercaptopropionic acid shows mercaptopropionic acid derivatives most stable[5]
- Pre-column optimized for low picomole amounts[6]
- Optimized reaction conditions and studied on-column stability of OPA-AA derivatives[7]
- Pre-column derivatization of sheep plasma, animal feeds and tissues[8]

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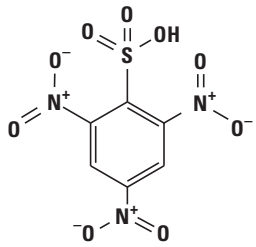
### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 26015 | Fluoraldehyde o-Phthalaldehyde Crystals | 5 g |
| 20150 | Brij-35 Detergent (30% w/w solution) | 950 ml |

## TNBSA

*An excellent choice for spectrophotometric detection.*



**TNBSA**
MW 293.17

Trinitrobenzene sulfonic acid (TNBSA) reacts readily with the primary amino groups of amino acids in aqueous solutionat pH8 to form yellow adducts. No colored derivatives are formed with the secondary amino acids praline and hydroxyproline. The colored derivatives are monitored at 345 nm and have extinction coefficients in the range of 1-1.5 x $10^4$.

TNBSA has been used as a hydrophilic modifying reagent for the detection of primary amines in samples containing amino acids, peptides or proteins. It is an excellent reagent for rapid qualitative and quantitative estimation of these biomolecules.

### Highlights:

- Couples with primary amines, sulfhydryls and hydrazides in aqueous solution at pH 8, without undesirable side reactions
- Excellent for solution or solid phase analysis
- Chromogenic ($\lambda_{max}$ = 335 nm)

**References**
1. Goodwin, J.F. and Choi, S-Y. (1970) *Clinical Chemistry.* **16,** 24-31.
2. Snyder, S.L. and Sobocinski, P.Z. (1975) *Anal Biochem.* **64,** 284-288.
3. Drozdovskaya, N.R., et al. (1982) *FEBS Lett.* **150,** 385
4. Takahashi, S., *et al.* (1984) *Chem. Lett.* (Jpn). **1,** 127

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 28997 | TNBSA (2,4,6-Trinitrobenzene sulfonic acid; 5% w/v methanol solution) | 100 ml |

# Introduction to Thermo Scientific Pierce
# Ninhydrin Detection Reagent for Amino Acids

Ninhydrin-based monitoring systems are among the most widely used methods for quantitatively determining amino acids after they are separated by ion exchange chromatography.

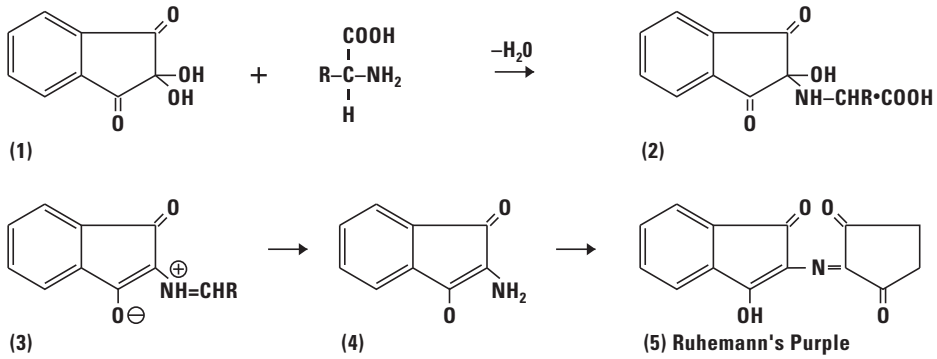
The color reaction between amino-containing compounds and ninhydrin (2,2-dihydroxy-1,3-indandione) is very sensitive. McCaldin has studied all phases of ninhydrin chemistry and proposed a mechanism for the reaction of ninhydrin with amino acids, accounting for the aldehydes, carbon dioxide, ammonia and hydrindantin known to be produced.[1] A yellow colored product (monitored at 440 nm) is formed upon reaction with the secondary amino acids, proline and hydroxyproline.[2] Ninhydrin decarboxylates and deaminates the primary amino acids, forming the purple complex known as Ruhemann's Purple,[3] which absorbs maximally at 570 nm.

Ninhydrin chemistry was adapted to a fully automatic, two-column amino acid analysis procedure in 1958 by Spackman, Stein and Moore.[4] Moore and Stein defined the requirements for a reducing agent (such as stannous chloride) to achieve reproducible color values for amino acids monitored with ninhydrin.[5] Titanous chloride was reported by James to eliminate precipitates encountered when using stannous chloride.[6-8] Methyl Cellosolve®(ethylene glycol monomethyl ether) buffered with 4 M sodium acetate at pH 5.51,[9] and dimethylsulfoxide (DMSO) buffered with 4 M lithium acetate at pH 5.20[10] are the most common solvents used for ninhydrin. DMSO remains stable longer than Methyl Cellosolve, particularly when kept chilled. These ninhydrin reagent solutions, with increased stability, were also reported by Kirschenbaum.[11] Moore called for substituting Methyl Cellosolve with, substituting sodium acetate with lithium acetate and including hydrindantin. The role of hydrindantin in determining amino acids was examined by Lamonthe and McCormick.[12]

Sensitivity of the ninhydrin system depends on several factors. Amino acids produce slightly different color yields, and these values may vary from one reagent preparation to the next. Ninhydrin also is sensitive to light, atmospheric oxygen and changes in pH and temperature. When ninhydrin becomes oxidized, its color does not develop well at 570 nm, but absorption at 440 nm remains fairly constant. When the height of the proline peak at 440 nm approaches the height of the glutamic acid peak at 570 nm, for equal amounts of each, reagent degradation is indicated.


**References**

1. McCaldin, D.J. (1960). *Chem. Rev.* **60**, 39.
2. Hamilton, P.B. (1966). *Advan. Chromatogr.* **2**, 3.
3. Ruhemann, J. (1911). *J. Chem. Soc. London* **99**, 797.
4. Spackman, D.H., Stein, W.H. and Moore, S. (1958). *Anal. Chem.* **30**, 1190.
5. Moore, S. and Stein, W.H. (1954). *J. Biol. Chem.* **211**, 907.
6. James, L.B. (1971). *J. Chromatogr.* **59**, 178.
7. James, L.B. (1978). *J. Chromatogr.* **152**, 298-300.
8. James, L.B. (1984). *J. Chromatogr.* **284**, 97-103.
9. Moore, S. and Stein, W.H. (1948). *J. Biol. Chem.* **176**, 367.
10. Moore, S. (1968). *J. Biol. Chem.* **243**, 6281-6283.
11. Kirschenbaum, D.M. (1965). *Anal. Biochem.* **12**, 189.
12. Lamonthe, P.J. and McCormick, P.G. (1973). *Anal. Chem.* **45**, 1906-1911.

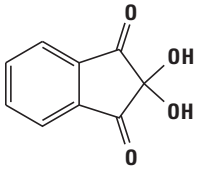




**Reaction Scheme.** The course of the ninhydrin reaction with amino acids is as follows:

1. Ninhydrin (2,2-dihydroxy-1,3-indandione) reacted with amino acid.
2. The intermediate formed as the first reaction product.
3. Intermediate gives rise to dipolar ion by decarboxylation and dehydration.
4. The dipolar ion hydrolyzes, producing the amine.
5. The amine condenses with a second molecule of ninhydrin to give Ruhemann's Purple.

## Ninhydrin

*The reagent of choice for detection of amino acids.*




**Ninhydrin**
MW 178.14

Since Stein and Moore pioneered amino acid chromatography in 1949,[1] Thermo Scientific Pierce Ninhydrin has been used in amino acid chromatography advancements. The most recent techniques and sensitive instruments require the superb color response and a low blank that only our Ninhydrin offers.

Our Ninhydrin is indefinitely stable and requires no refrigeration or special care. Just keep the bottle tightly sealed, avoiding direct sunlight and ammonia in the laboratory atmosphere.

**References**

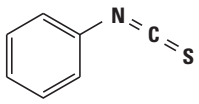
1. Stein, W.H. and Moore, S. (1949). Cold Spring Harbor Symp. *Quant Biol.* **14**, 179.
2. Moore, S. (1968). Amino acid analysis: aqueous dimethyl sulfoxide as solvent for the ninhydrin reaction. *J. Biol. Chem.* **243(23)**, 6281-6283.
3. James, L.B. (1978). Amino acid analysis: ninhydrin reaction with titanous chloride. *J. Chromatogr.* **152**, 298-300.

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **21003** | Ninhydrin | 500 g |

## PITC (Phenylisothiocyanate)

*Ideal for the quantitative pre-column derivatization of amino acids by reverse-phase HPLC.*[14]



**PITC**
**Edman's Reagent**
MW 135.19

Thermo Scientific Pierce PITC, also known as Edman's Reagent, reacts readily with amino acids in 5-10 minutes at room temperature. The resulting phenylthiocarbamyl derivatives can be separated and quantified in 30 minutes using reverse-phase HPLC. This method produces stable products with all amino acids, including proline.

### TO COUPLE AMINO ACID STANDARD H WITH PITC.[1]

1. Dry 10 µl Pierce Amino Acid Standard H in a small test tube. Dissolve dried standard in 100 µl coupling buffer (acetonitrile: pyridine: triethylamine: $H_20$, 10:5:2:3).
2. Dry standard solution by rotary evaporation. Dissolve the residual amino acids once more in 100 µl of coupling buffer.
3. Add 5 µl of Pierce PITC.
4. Allow a 5-minute reaction at room temperature.
5. Evaporate sample to dryness by rotary evaporation under high vacuum.
6. Dissolve the resulting PITC-amino acids in 250 µl of 0.05 M ammonium acetate, water or water:acetonitrile (7:2).
7. Analyze quantities of 1 to 10 µl (100 to 1,000 picomoles of each amino acid) by reverse-phase HPLC.

**NOTE:** Make certain that all HCl is evaporated before derivatization.

**References**
1. Heinrikson, R.L. and Meridith, S.C. (1984). *Anal. Biochem.* **136**, 65-74.
2. Scholze, H. (1985). *J. Chromatogr.* **350**, 453-460.
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4. Evert, R.F. (1986). *Anal. Biochem.* **154**, 431-435.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 26922 | PITC (Phenylisothiocyanate) | 10 x 1 ml ampules |
| 20088 | Amino Acid Standard H | 10 x 1 ml ampules |

✖*Additional hazardous handling charge.*

## Fluoraldehyde Reagent Solution

*Our ready-to-use OPA derivatization reagent for highly sensitive amino acid analysis*

Ready-to-use Thermo Scientific Fluoraldehyde Reagent Solution is a pre-column amine derivatization reagent that is suitable for amino acid analysis by HPLC.

At room temperature, Fluoraldehyde Reagent Solution reacts rapidly with primary amines, and it can be injected into your LC with no further processing.

Our Fluoraldehyde Reagent Solution is ideal for reverse-phase HPLC because the derivatives formed are less polar than free amino acids. All primary amino acids react with Fluoraldehyde Reagent, resulting in highly fluorescent isoindole derivatives.

### TO COUPLE AMINO ACID STANDARD H WITH OPA:

1. Mix 5-10 µl of Pierce Amino Acid Standard H with 5 µl of Pierce Fluoraldehyde Reagent Solution.
2. After one minute, add 20-100 µl of 0.1 M, pH 7.0 sodium acetate to standard solution. Mix.
3. Subject a 20 µl aliquot for analysis by reverse-phase HPLC.

**NOTE:** For optimal 'reproducibility, maintain constant reaction times.

### Highlights:

- For pre-column monitoring of amino acid utilization in cell culture[1]
- For hydrolyzate and physiological amino acid derivatization[2]
- Optimal reaction conditions are used to study the on-column amino acid stability[3]
- For hypochlorite oxidation of secondary amino acids[4]
- For pre-column analysis of urinary amino acids[5]
- For use with ethane thiol for improved derivative stability[6,7]
- An oxidation method for proline and secondary amino acids prior to derivatization[8]

**References**
1. Seaver, S.S., *et al.* (Sept./Oct. 1984). *BioTechniques*, 254-260.
2. Jones, B.N., (1983). *J. Chromatogr.* **266**, 471-482.
3. Cooper, J.D.H., *et al.* (1984). *Anal. Biochem.* **142**, 98-102.
4. Bohlen, P., *et al.* (1979). *Anal. Biochem.* **94**, 313-321.
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7. Hill, D.W., *et al.* (1979). *Anal. Chem.* **51**, 1338-1341.
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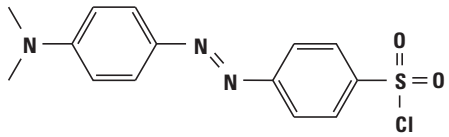
10. Gardner, W.S. and Miller III, W.H. (1980). *Anal. Biochem.* **101**, 61-65.
11. Jones, B.N. and Gilligan, J.P. (1981). *J. Liq. Chromatogr.* **4**, 564-586.
12. Dong, M.W. and DiCesare, J.L. (1983). *LC* **1**, 222-228.
13. Jones, B.N. and Gilligan, J.P. (1983). *An. Biotech. Lab.*, December, 46-51.
14. Bhown, A.S., *et al.* (1983). *LC* **1**, 50-52.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 26025 | Fluoraldehyde Reagent Solution (o-Phthalaldehyde) | 945 ml |
| 26015 | Fluoraldehyde OPA Crystals | 5 g |
| 20088 | Amino Acid Standard H | 10 x 1 ml ampules |

## Dabsyl Chloride

*It's recrystallized twice for twice the quality!*



**Dabsyl Chloride**
MW 323.80

Thermo Scientific Pierce Dabsyl Chloride is for the pre-column derivatization and detection of amino acids in visible light down to sub-picomolar levels, followed by reverse-phase HPLC.

### Highlights:

- Analysis of 10-30 ng of protein hydrolyzates[1,2]
- Analysis of peptides and determination of C-terminal sequence of polypeptides[1]
- Analysis of phospho-amino and amino acid amides[3]
- Analysis of amino acid neurotransmitters in mouse brain[4]
- Optimal reaction conditions[5]

**References**
1. Chang, J.Y., *et al.* (1981). *Biochem. J.* **199**, 547-555.
2. Chang, J.Y., *et al.* (1982). *Biochem. J.* **199**, 803-806
3. Chang, J.Y. (1984). *J. Chromatogr.* **295**, 193-200.
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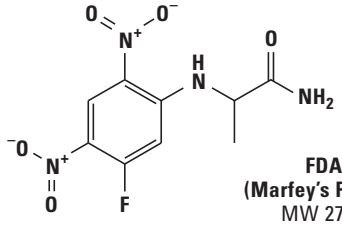
5. Vendrell, J., *et al.* (1986). *J. Chromatogr.* **358**, 401-413.
6. Lin, J.K., *et al.* (1980). *Clin. Chem.* **26**, 579-583
7. Chang, J.Y., *et al.* (1983). *Methods. Enzymol.* **92**, 41-48.
8. Stocchi, V., *et al.* (1985). *J. Chromatogr.* **349**, 77-82.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 21720 | **Dabsyl Chloride (4-Dimethylaminoazobenzene-4-Sulfonyl Chloride)** | 500 mg |

## FDAA, Marfey's Reagent

*Derivatizes optical isomers of amino acids in just 90 minutes.*



**FDAA**
**(Marfey's Reagent)**
MW 272.19

Thermo Scientific Pierce FDAA, (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide), offers complete derivatization of amino acid isomers in 90 minutes. Derivatized amino acids then are separated and quantitated by reverse-phase HPLC. The nature of the reagent and the the resultant reaction products with D-diastereomers suggest that strong intramolecular hydrogen bonding causes these derivatives to elute much later than their L-diastereomer counterparts. Derivatives have an absorption coefficient of approximately $3 \times 10^4$. They can be detected by UV at 340 nm with picomole sensitivity. Complete instructions are included with each order.

### TO PREPARE FDAA DERIVATIVES:

1. Place 100 µl (5 µmoles) sample in a 1.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Add 200 µl of 1% (w/v) solution of Pierce FDAA in acetone. Add 40 µl of 1.0 M sodium bicarbonate (µmoles FDAA: µmoles amino acid should be 1.5:1.0.)
3. Heat at 40°C for 1 hour. Remove and cool.
4. Add 20 µl 2 M HCl. Allow sample to degas.
5. Analyze.
   Conditions: Spheri-5, RP-18, 10 cm x 4.6 cm
   UV at 340 nm
   A: 0.05 M TEA phosphate pH 3.0
   B: $CH_3CN$
   Linear Gradient, 10% B to 40% B in 45 minutes
   Flow: 2.0 ml/min. at 25°C

**References**
1. Marfey, P., *et al.* (1984). *Carlsberg Res. Comm.* **49**, 585-590.
2. Marfey, P. (1984). *Carlsberg Res. Comm.* **49**, 591-596.
3. Szókán, G., *et al.* (1988). Applications of Marfey's Reagent in racemization studies of amino acids and peptides. *J. Chromatogr.* **444**,115-122.
4. Aberhart, D.J., *et al.* (1985). Separation by high-performance liquid chromatography of (3R)- and (3S)-B-Leucine as diastereomeric derivatives.[1] **151**, 88-91.
5. Martinez del Pozo, *et al.* (1989). Stereospecificity of reactions catalyzed by bacterial D-amino acid transaminase. *J. Biol. Chem.* **264(30)** 17784-17789.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 48895 | **FDAA Marfey's Reagent (1-fluoro-2, 4-dinitrophenyl-5-L-alanine amide)** | 50 mg |

## Ultra-Pure Solvents for Amino Acid Analysis

*Ideal for HPLC and spectrophotometric applications.*

Thermo Scientific Pierce HPLC Grade Solvents and Water are specially purified by proprietary methods and tested to ensure lot-to-lot consistency with a low UV absorbance to provide you with the most sensitive detection across all wavelengths. All are packaged in amber glass bottles and sealed with Teflon TFE-lined fluorocarbon caps for ultimate protection. Our solvents are then tested to the highest specifications to ensure the integrity of your data, maximize sensitivity in your assay and prolong the life of your equipment.

### Physical Properties

#### Acetonitrile, HPLC Grade

- UV Cutoff: 190 nm
- Optical Absorbance: <0.02 at 220 nm
- Refractive Index at 25°C: 1.342

#### Water, HPLC Grade

- UV Cutoff: 190 nm
- Optical Absorbance: <0.005 at 220 nm
- Refractive Index at 25°C: 1.332

#### Dimethylformamide (DMF), Sequencing Grade

- HCON $(CH_3)_2$
- Purity (GC): ≥99%
- MW: 73.09
- Density: 0.944
- B.P. 153°C
- Water: <0.1%

#### Dimethylsulfoxide (DMSO), Sequencing Grade

- $C_2H_6OS$
- Purity (GC): >99.5%
- MW: 78.13
- Density: 1.101
- Water: ≤0.2%

#### Pyridine

- $C_5H_5N$
- Purity (GC): ≥99%
- MW: 79.10
- Density: 0.978
- B.P.: 115°C

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖51101 | Acetonitrile | 1 L |
| 51140 | Water | 1 L |
| ✖20673 | Dimethylformamide (DMF) | 50 ml |
| ✖25104 | Pyridine ($C_5H_5N$) | 100 g |
| 20688 | Dimethylsulfoxide (DMSO) | 950 ml |

✖*Additional hazardous handling charge.*
*For GC Grade DMF and DMSO, see page 18.*

# Introduction to **Sample Handling**

## Sample Handling

**To complete our offering for gas and liquid chromatography, we offer a wide range of heating and stirring systems, vials, closures and laboratory cleaners. All Thermo Scientific sample handling products are manufactured under strict conditions, providing the quality you need for reliable derivitization reactions.**

## Reacti-Vial Small Reaction Vials

*Make small-sample handling easy and convenient.*

**Ideal for:**

- Residue isolation
- Derivative preparation
- Maximum sample retrieval
- Moisture protection
- Sample storage

Thermo Scientific Reacti-Vial Small Reaction Vials have an internal cone designed to make small-sample collection and handling easy and convenient. The cone feature is particularly useful for removing small quantities of sample with a syringe, even into the microliter range. The extra thick glass wall magnifies the sample, making these units ideal for observing chemical reactions. Reacti-Vial Small Reaction Vials can he used for derivatization, isolation and purification. You can also use Reacti-Vial Small Reaction Vials for precipitations, centrifugations and solvent separations.

Our amber Reacti-Vial Small Reaction Vials are manufactured from amber glass, and are amber throughout. These amber vials assure that your light-sensitive compounds are well protected. All Pierce Reacti-Vial Small Reaction Vials are supplied complete with Open-Top Screw Caps and Teflon/Rubber Laminated Discs (other discs can be ordered separately, see optional accessories on right).

## Reacti-Vial Small Reaction Vials

| Size | Dimensions (Diam. x Height) (mm ± 1mm) | Inside Diameter (mm) | Thread Style | Clear Pkg. of 12 Product # | Clear Pkg. of 12 U.S. Price | Amber Pkg. of 12 Product # | Amber Pkg. of 12 U.S. Price |
|---|---|---|---|---|---|---|---|
| 100 µl | 12 x 32 | 8 | 425-8 | **13100** | **$ 85** | | |
| 0.3 ml | 13 x 32 | 11 | 425-13 | **13220** | **$ 81** | | |
| 1.0 ml | 13 x 45 | 11 | 425-13 | **13221** | **$105** | **13097** | **$103** |
| 3.0 ml | 20 x 47 | 18 | 425-20 | **13222** | **$110** | | |
| 5.0 ml | 20 x 60 | 18 | 425-20 | **13223** | **$115** | **13099** | **$140** |
| 10.0 ml | 25 x 69 | 22 | 425-24 | **13225** | **$218** | | |

## Optional Accessories

| Vial Size | Teflon/ Silicone Discs Pkg. of 72 Product # | Teflon/ Silicone Discs Pkg. of 72 U.S. Price | Rubber Laminated Discs Pkg. of 72 Product # | Rubber Laminated Discs Pkg. of 72 U.S. Price | Open-Top Screw Caps Pkg. of 72 Product # | Open-Top Screw Caps Pkg. of 72 U.S. Price | Miniert Valves Pkg. of 72 Product # | Miniert Valves Pkg. of 72 U.S. Price | Reacti-Vial Magnetic Stirrers Pkg. of 6 Product # | Reacti-Vial Magnetic Stirrers Pkg. of 6 U.S. Price |
|---|---|---|---|---|---|---|---|---|---|---|
| 100 µl | **12708** | **$ 45** | | | **13208** | **$ 29** | | | | |
| 0.3 ml | **12712** | **$ 57** | **12412** | **$ 44** | **13215** | **$ 29** | | | **16010** | **$100** |
| 1.0 ml | **12712** | **$ 57** | **12412** | **$ 44** | **13215** | **$ 29** | | | **16010** | **$100** |
| 3.0 ml | **12718** | **$ 85** | **12418** | **$ 50** | **13218** | **$ 32** | **10135** | **$129** | **16000** | **$102** |
| 5.0 ml | **12718** | **$ 85** | **12418** | **$ 50** | **13218** | **$ 32** | **10135** | **$129** | **16000** | **$102** |
| 10.0 ml | **12722** | **$103** | **12422** | **$ 57** | **13219** | **$ 29** | **10130** | **$132** | **16000** | **$102** |

*For more information about discs and Mininert Valves, see page 58, 59.*

*For more information about Reacti-Vial Magnetic Stirrers, see page 65.*

## Discs and Septa for Hypo-Vial Storage Vials

*A variety of choices to fit your exact needs.*

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 12720 | Teflon®/ Silicone Discs | Pkg. of 72 |
| 13050 | Gray Butyl Septa | Pkg. of 72 |
| 13230 | Gray Hycar Septa | Pkg. of 72 |
| 13237 | Silicone Septa | Pkg. of 72 |

**For disc and septum chemical compatibility guide, see page 59.*

## Pre-Cleaned Vials

*Versatile Thermo Scientific Pierce Pre-Cleaned Vials for your sampling, analytical and general laboratory needs.*

### Advantages

- Allow instant syringe access to reagents and standards through the open top screw caps
- Long-term sample storage of biological media and volatile solutions
- Ideal for sample collection and testing

Our Sample Storage Vials provide a meticulously cleaned, ready-to-use vial system. Because they contain no residue from the manufacturing process, you can eliminate the inconsistency, inconvenience and expense of cleaning and drying sampling and storage vials.

Thermo Scientific Cap Liners compress to tightly seal your sample. Thermo Scientific Discs are autoclavable and resealable. The silicone body (90 mils thick) and the Teflon Face (10 mils) are structurally not cemented like most cap liners. So, no cement can be leached or baked out after needle penetration. The Teflon Face provides an inert barrier between your sample and the screw cap.

Pierce Vials are washed, dried and shrink-wrapped for contaminant-free shipping. Each package consists of 72 fully assembled screw cap vials and Teflon/Silicone Discs in convenient-to-use, shrink-wrapped divider trays.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 13504 | Pre-Cleaned Vials | 3.5 ml, clear, pkg. of 72 |
| 13510 | Pre-Cleaned Vials | 40.0 ml, clear, pkg. of 72 |

## Hypo-Vial™ Sample Storage System

*Made of high-quality borosilicate glass for heating or autoclaving.*

Thermo Scientific Hypo-Vial Sample Storage Vials for storage and handling of chemical materials includes high-quality amber or clear borosilicate glass vials in a variety of sizes, a selection of discs and septa that are compatible with a wide range of materials and easy-to-use tools for applying and removing seals.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 12944 | Hypo-Vial Sample Storage Vials | 30.0 ml, clear, Pkg. of 72 |
| 12969 | Hypo-Vial Sample Storage Vials | 50.0 ml, clear, Pkg. of 72 |
| 12995 | Hypo-Vial Sample Storage Vials | 125.0 ml, clear, Pkg. of 84 |

## Pre-Cleaned Water Sampling Vials

*We pre-clean our vials to save you time and hassle.*

### 40 ml EPA* Water Sample Kits

For sampling to analyze priority pollutants (including trihalomethanes), Thermo Scientific Pierce Pre-cleaned Water Sampling Vials are a great time-saver. Ready for immediate use, our Pre-Cleaned Water Sampling Vials eliminate the time, inconvenience and expense of cleaning and drying your sampling vials. Each Pierce 40 ml Vial package includes 72 pre-cleaned, fully assembled 40 ml Screw Cap Septum Vials. (Product #13075) and Teflon/Silicone Discs (Product #12722) in convenient-to-use shrink-wrapped divider trays.

**Pre-cleaned for discrete water sampling according to EPS 40 CFR 136 "Guidelines for Establishing Test Procedures for the Analysis of Pollutants" and EPA 40 CFR 141 "National Interim Primary Drinking Water Regulations; Control of Trihalomethanes in Drinking Water."*

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 13510 | 40 ml Pre-Cleaned Water Sampling Vials | Pkg. of 72 |

## Screw Cap Septum Vials

*Autoclavable, borosilicate glass available in clear or amber.*

- Sample collection - the 40 ml clear or amber vial with a Thermo Scientific Disc is suitable for discrete water sampling under EPA 40 CFR Parts 136 and 141
- Automated GCs and LCs - the 1.5 ml clear and amber vials fit autosamplers using standard 12 x 32 mm vials
- Heavy-duty, flip-top divider box provides easy access to vials; caps and septa and offers a convenient sample storage center

Thermo Scientific Pierce Screw Cap Septum Vials are supplied complete with Open-Top Screw Caps.

For economy, convenience and versatility in a vial and closure system, our Screw Cap Septum Vials are your best choice. A wide assortment of special closures and accessories make this system perfect for:

- Storage of reagents and standards under complete seal with instant syringe access
- Small derivatization reactions

### Screw Cap Septum Vials

| Size | Dimensions (Diam. x Height) (mm ± 1mm) | Inside Diameter (mm) | Thread Style | Clear Pkg. of 12 Product # | Clear Pkg. of 12 U.S. Price | Amber Pkg. of 12 Product # | Amber Pkg. of 12 U.S. Price |
|---|---|---|---|---|---|---|---|
| 1.5 ml | 12 x 32 | 8 | 425-8 | | | **13080** | **$73** |
| 3.5 ml | 15 x 45 | 12 | 425-13 | **13019** | **$ 73** | | |
| 7.0 ml | 17 x 60 | 13 | 425-15 | **13028** | **$ 83** | | |
| 14.0 ml | 21 x 70 | 16 | 425-18 | **13043** | **$ 98** | | |
| 40.0 ml | 27 x 95 | 22 | 425-24 | **13075** | **$128** | | |

### Optional Accessories

| Vial Size | Teflon/ Silicone Discs Pkg. of 72 Product # | Teflon/ Silicone Discs Pkg. of 72 U.S. Price | Teflon/ Rubber Laminated Discs Pkg. of 72 Product # | Teflon/ Rubber Laminated Discs Pkg. of 72 U.S. Price | Mininert Valves Pkg. of 72 Product # | Mininert Valves Pkg. of 72 U.S. Price | Open-Top Screw Caps Pkg. of 72 Product # | Open-Top Screw Caps Pkg. of 72 U.S. Price |
|---|---|---|---|---|---|---|---|---|
| 1.5 ml | **12708** | **$45** | | | | | **13208** | **$29** |
| 3.5 ml | **12712** | **$57** | **12412** | **$44** | | | **13215** | **$29** |
| 7.0 ml | **12713** | **$65** | | | | | **13215** | **$31** |
| 14.0 ml | **12716** | **$82** | | | | | **13218** | **$32** |
| 40.0 ml | **12722** | **$103** | **12422** | **$57** | **10130** | **$132** | **13218** | **$32** |

*For disc and discs and Mininert Valves, see page 58, 59.*

## Teflon/Silicone Discs

*Unique discs that combine the inertness of a Teflon coating with the resealability of silicone.*

Autoclavable Thermo Scientific Teflon/Silicone Discs are specifically designed to combine the resealability of silicone with the inertness of a Teflon coating. Many sizes are available to fit our Hypo-Vial and Reacti-Vial Screw Cap.

Structurally bonded (not cemented) Teflon coating to silicone. No cement to be leached or baked out of your sample after needle penetration.

### Highlights:

- Reseals instantly puncture after puncture
- Compresses, giving it a "lock-washer" effect in maintaining a tight seal, and forces the Teflon coating to conform to the sealing surface
- No bent needles from a septum that is too hard; standard syringe and CC needles penetrate the entire disc with ease
- Protection of Teflon coating: The Teflon layer is a full 10 mils thick

### Thermo Scientific Teflon/Silicone Discs (pkg. of 72)

| Product # | Silicone Thickness (mils) | Teflon Thickness (mils) | Fits These Containers |
|---|---|---|---|
| **12708** | 75 | 10 | 100 µl Reacti-Vial Small Reaction Vials<br>1.5 ml Screw Cap Septum Vials |
| **12712** | 90 | 10 | 0.3 and 1.0 ml Reacti-Vial Small Reaction Vials<br>3.5 ml Screw Cap Septum Vials |
| **12713** | 90 | 10 | 7 ml Screw Cap Septum Vials |
| **12716** | 90 | 10 | 14 ml Screw Cap Septum Vials |
| **12718** | 90 | 10 | 3 and 5 ml Reacti-Vial Small Reaction Vials |
| **12720** | 125 | 10 | 6-125 ml Hypo-Vial Sample Storage Vials |
| **12722** | 90 | 10 | 25 and 40 ml Screw Cap Septum Vials<br>10 ml Reacti-Vial Small Reaction Vials |

## Teflon/Rubber Laminated Discs

*For a highly inert and nonreactive seal.*

Thermo Scientific Teflon/Rubber Laminated Discs are constructed of white pharmaceutical rubber with 5 mils of Teflon coating bonded to one side. Total thickness of the disc is approximately 60 mils. The discs are excellent for use as cap liners when a highly inert nonreactive seal is desired.

Our rigid Teflon/Rubber Laminated Discs are more difficult to puncture than Teflon/Silicone Discs. Consequently, care must be taken when puncturing these discs to avoid bending the needle.

Our Teflon/Rubber Laminated Discs are auto-clavable and demonstrate no loss of integrity after heating above 100°C for 5 hours.

### Thermo Scientific Teflon/Rubber Laminated Discs (pkg. of 72)

| Product # | Size | Fits These Containers |
|---|---|---|
| **12412** | 12 mm | 0.3 and 1.0 ml Reacti-Vial Small Reaction Vials,<br>3.5 ml Screw Cap Septum Vials |
| **12418** | 18 mm | 3 and 5 ml Reacti-Vial Small Reaction Vials |
| **12422** | 22 mm | 25 and 40 ml Screw Cap Septum Vials |

## Mininert Valves

*Ideal for chemicals that deteriorate or evaporate in conventionally sealed containers.*

They're easy to use. Push the green button to open, insert syringe needle and take sample, withdraw needle, then push red button to close. To change needle-seal septa, simply push the old septum out with a 1/8" diameter rod and push a new cylinder septum in. This is done with the valve closed to prevent exposure of contents.

Thermo Scientific Mininert Push-button Valves are highly dependable leak-tight closures for Screw Cap Septum Vials and other laboratory containers. Constructed of chemical-resistant Teflon coating, the valves provide an inert, high-pressure seal.

Mininert Valves are a superior replacement for rubber septum stoppers and ordinary screw caps. You can easily access the contents by inserting a syringe needle. A rubber gasket above the Teflon coating valve stem provides a seal for the needle when the valve is open. The seal prevents leakage and exposure of the contents during sampling.

**Mininert Valves are unique and practical seals for these containers:**

- Screw Cap Septum Vials
- Thermo Scientific Hypo-Vial Sample Storage Vials
- Thermo Scientific Reacti-Vial Small Reaction Vials

## Ordering Information

| Product # | Description | Fits these Containers | Size | Pkg. Size |
|---|---|---|---|---|
| **10135** | Mininert Valves | 3 and 5 ml Reacti-Vial Small Reaction Vials | 20 mm | 12/pkg |
| **10130** | Mininert Valves | 40 ml Screw Cap Septum Vials | 27 mm | 12/pkg |

## Disc and Septa Compatibility Guide

| Closure Type | Resealability | Recommended For Use With | Not Recommended For Use With |
|---|---|---|---|
| Teflon/Silicone Discs | Excellent | DMF, DMSO, organic solvents, pyridine, THF and silylation reactions | Strong corrosives, such as chlorosilanes |
| Teflon/Rubber Laminated Discs | Poor | Corrosives such as chlorosilanes, DMF, DMSO, organic solvents, pyridine and THF | Trifluoracetic anhydride |
| Butyl Rubber Septa | Good | Acetic acid, acetic anhydride < 50%, acetone, acetonitrile, alcohols, amines, carbon dioxide, diethylamine, DMF, DMSO, ethanolamine ethylacetate (for short-term use), phenol & water | TEA triethylamine, alkanes, benzene, carbon disulfide chlorinated solvents, cyclohexane, ethylacetate for long-term use, fuels, heptane, and hexane |
| Hycar Septa | Good | Chlorinated organic compounds, hydrocarbons such as heptane and hexane | Acetonitrile, benzene, chloroform, DMF, DMSO, pyridine, THF and toluene |
| Silicone Septa | Excellent | Acetone, alcohols, DMF, DMSO, ether, some other ketones and water | Acetonitrile, benzene, chloroform, heptane, hexane, pyridine, THF and toluene |

## PCC-54 Detergent Concentrate

*Offers safe and effective cleaning in a convenient pump-handle dispenser!*

**PCC-54 Concentrate offers these unique features:**

- Rinses clean, providing film-free surfaces
- Safe to handle
- Economical
- Trouble-free disposal

Thermo Scientific PCC-54 Concentrate is a special detergent formulation designed for efficient cleaning of glass, plastic, porcelain and ferrous metal surfaces. PCC-54 Concentrate can be used in all applications in which ultimate cleanliness of apparatus and equipment is essential. It removes stubborn contaminants and rinses completely, leaving your surface free of any annoying residues or films.

PCC-54 Concentrate is an effective replacement for chromic acid or other harsh cleaning solutions - eliminating harm to you, your clothing or your apparatus (see comparison chart below).

Only we offer PCC-54 Concentrate in an exclusive three-liter pump dispenser bottle. Sparkling clean labware is just a pump away.

**Here's how easy sparkling clean labware can be (instructions for normal use):**

1. Pump 20cc of PCC-54 Concentrate and add to 1 liter of water at 50°C.
2. Immerse object completely into solution.
3. Incubate at least 30 minutes.
4. Immediately after cleaning, rinse with tap water, then with distilled water.

### Ordering Information

| Number | Description | Pkg. Size | Volume (12% Solution) |
|---|---|---|---|
| 72288 | PCC-54 Detergent Concentrate | 3 L | 150 L |
| 72289 | PCC-54 Detergent Concentrate | 4 x 3 L | 600 L |
| 72290 | PCC-Pfree Phosphate Free Detergent Concentrate | 3 L | 150 L |
| 72300 | Pump Dispenser (Available only upon request) | 1 | |

**Comparison of Thermo Scientific Cleaning Agents to chromic acid for effectiveness and safety**

| PCC-54 & RBS Cleaning Agents | Chromic Acid |
|---|---|
| Moderately alkaline | Strongly acidic |
| Harmless to skin and clothing | Extremely corrosive to skin and clothing |
| No disposal problem; Causticity is the same as .001 N sodium hydroxide solution | Disposal difficulties due to corrosive nature |
| No etching of glassware | Frequently etches glassware |
| Rinses tree at all residue | Occlusion of chromic salts |
| Works rapidly when boiled; It using cold solution, action can be accelerated by increasing concentration | Slow acting |
| Acts by solubilizing and emulsifying contaminants with no destructive chemical action likely to yield gaseous toxic radioactive compounds | Cleans by oxidation. Compounds containing radioactive Cl, F, S, N or C atoms may yield radioactive gases, producing respiratory hazards |
| Easily removes difficult substances such as tars, distillation residues, silicone, oils, etc. | Has only a slight effect on these materials |

## RBS Cleaning Agents

*This versatile, safe alternative to chromic acid even inactivates the HIV virus!*


**Useful for:**

- General laboratory work
- Optical applications - slides, lenses, mirrors, reflectors, etc.
- Radiochemical decontamination
- Food and beverage equipment sanitation
- Electronics - ultimate cleanliness without residue

Thermo Scientific RBS Cleaning Agents are mildly basic surfactant concentrates that will not harm you or your clothing. They are a mixture of anionic and nonionic detergents capable of removing both organic and inorganic materials. Glass, plastics, quartz, porcelain and ferrous metal apparatus can be cleaned safely and effectively in a 2% working solution of the concentrate or in a 0.2% solution of the solid. These easy-to-use cleaning agents work by penetrating contaminated substances and the surface to be cleaned.

Because our RBS Cleaning Agents are nonflammable and noncorrosive, they are easy to handle and can be disposed of safely The table on the previous page compares Thermo Scientific cleaning agents with the effective, but hazardous, chromic acid cleaning solution. Our cleaning agents are superior to chromic acid in every category.

RBS Cleaning Agents prepared with ethanol or methanol are safer for both the labware and the user than the caustic alcohol solutions commonly used to remove tars, distillation heels and silicone greases. The alcohol/RBS Agent combination removes tenacious substances from glassware without worry about caustic bums or etching of the glass surfaces.

The Pasteur Institute of Paris, France demonstrated that RBS-35 Detergent even inactivates the HIV virus. The virus was inactivated after 5 minutes in contact with a 4% solution of the RBS®-35 Detergent.

Our RBS Cleaning Agents are available in a variety of forms to meet your cleaning needs.

**RBS-35 Detergent Concentrate** is a biodegradable, general purpose lab cleaner that allows for long soaking without leaving deposits. It decontaminates radioactivity and removes protein, lipids, greases, oils and still residues.

In the past, pipettes and other laboratory equipment with hard-to-clean crevices have been soaked in hazardous chemicals such as chromic acid. RBS-35 Detergent Concentrate offers a safe, effective alternative to chromic acid for these applications (see table on the previous page).

**RBS Detergent Solid** is a low-foaming, surface-active powder that is ideal for use in washing machines equipped with a powder dispenser. It is easily eliminated by rinsing, and it leaves no traces. Recommended concentration is 0.1-0.3%.

**RBS-pF Detergent Concentrate** was developed as a substitute for surfactants that contain phosphates. It is biodegradable and contains no phosphates, which can harm the environment when released into sewage systems.

### Ordering Information

| Product# | Description | Pkg. Size | Volume (2% Solution) |
|---|---|---|---|
| 27950 | RBS-35 Concentrate | 1 kg | 50 qt. |
| 27952 | RBS-35 Concentrate | 5 kg | 200 qt. |
| 27853 | RBS-35 Concentrate | 30 kg | 1,200 qt. |
| 27968 | RBS Solid | 5 kg | |
| 27959 | RBS-pF* Concentrate | 1 kg | 50 qt. |
| 27960 | RBS-pF* Concentrate | 5 kg | 200 qt. |

** pF = phosphate free*


## Reacti-Therm™ Dry Block Sample Incubation System

*Featuring our exclusive Reacti-Therm Modules for dry block heating - and no sample contamination!*

The Thermo Scientific Reacti-Therm System delivers uniform dry heat with unmatched convenience and versatility. The dry heat prevents many of the problems associated with water baths, including sample contamination.

**Reacti-Therm Modules** are easy-to-use, constant-temperature heaters that are ideal for your routine incubations. They also provide constant temperature control for samples held in Thermo Scientific Reacti-Vial Small Reaction Vials, as well as for samples in test tubes, microcentrifuge tubes and other small containers. Most applications that require heating, stirring or evaporation of small samples would benefit from the convenience and efficiency of Reacti-Therm Modules. These applications include:

- Sample incubation
- Sample evaporation
- Protein hydrolysis
- Small-scale reactions
- Vacuum hydrolysis for amino acid analysis
- Derivatization reactions for HPLC and GC

Our Reacti-Therm Modules transfer heat through an aluminum alloy block. They hold a wide variety of interchangeable Thermo Scientific Reacti-Block Aluminum Blocks (page 64-65). Choose from four module designs to meet your exact incubation needs.

Single-Block Reacti-Therm Heating Modules and Reacti-Therm Heating/Stirring Modules - now quicker than ever!

Single-block Reacti-Therm Heating Modules feature a solid state electronic control. This highly efficient control system allows faster and easier temperature settings.

### Dual Volt Modules

| Product # | Description | Pkg. Size |
|---|---|---|
| 18820 | Reacti-Probe Remote Temperature Probe | 1 unit |
| 18821 | Reacti-Therm Heating/Stirring Module (Single Block) *Available Fall 2008* | 1 unit |
| 18822 | Reacti-Therm Heating Module (Single Block) *Available Fall 2008* | 1 unit |
| 18823 | Reacti-Therm Heating/Stirring Module (Triple Block) *Available Fall 2008* | 1 unit |
| 18824 | Reacti-Therm III Heating Module (Triple Block) *Available Fall 2008* | 1 unit |
| 18825 | Reacti-Vap Evaporator | 1 unit |
| 18826 | Reacti-Vap Evaporator | 1 unit |

*Underwriters Laboratories, Inc. Listed*

*Note: Our Reacti-Therm Modules bear a CE marking for meeting the requirements of the European Union's Low-Voltage and EMC Directives.*

## Reacti-Vap Evaporators

*Sample evaporation made easy!*

*Thermo Scientific Reacti-Vap Evaporator (9-port) and Reacti-Vap III Evaporator (27-port).*

Thermo Scientific Reacti-Vap Evaporators are precision-machined gassing manifolds. They provide simple, efficient evaporation by allowing the simultaneous or separate delivery of nonreactive pressurized gas to samples.

The Reacti-Vap III Evaporator triples the number of samples you can evaporate. Nine needles attach to each of the three individually regulated chambers. The evaporating head tilts back for easy needle attachment and removal.

The standard Reacti-Vap Evaporator attaches easily to single-block Reacti-Therm Modules. The Reacti-Vap III unit attaches easily to Reacti-Therm III Modules.

### Ordering Information

| Product # | Description |
|---|---|
| **18825** | **Reacti-Vap Evaporator (9-port)**<br>For use with Reacti-Therm Single Block Modules; 18822 and 18821, *Available Fall 2008*<br>Includes 9 needles and plugs |
| **18826** | **Reacti-Vap III Evaporator (27-port)**<br>For use with Reacti-Therm III Modules; 18823 and 18824, *Available Fall 2008*<br>Includes 27 needles and plugs |

## Reacti-Vap Standard and Teflon Coated Needles

*Reduce cross-contamination and corrosion.*

Thermo Scientific Reacti-Vap Teflon Coated Needles are made exclusively for use in Reacti-Vap Evaporators. They are blunt-ended, 19-gauge, stainless steel needles that reduce cross-contamination and corrosion when evaporating solvents that contain strong acids.

Each Reacti-Vap Needle has a Luer-Lok hub for leak-proof attachment to Reacti-Vap Evaporators Needles are available in 4- and 6-inch lengths.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| **18782** | **Reacti-Vap Replacement Tube Kit**<br>2.5 inch (64 mm) | Pkg. of 9 and plugs |
| **18784** | **Reacti-Vap Teflon Coated Needles**<br>4-inch (102 mm) x 19 gauge | Pkg. of 9 |
| **18786** | **Reacti-Vap Teflon Coated Needles**<br>6-inch (152 mm) x 19 gauge | Pkg. of 9 |

## Reacti-Therm Thermometers

*Teflon-coated, designed specifically for dry incubations.*

### Ordering Information

| Product # | Description |
|---|---|
| 18914 | Reacti-Therm Thermometer, Mercury-free (0-100°C) |
| 18915 | Reacti-Therm Thermometer, Mercury-free (0-200°C) |

## Reacti-Block Aluminum Blocks

*There is one that is right for your sample needs!*

Thermo Scientific Reacti-Block Aluminum Blocks are available with many hole configurations, machine-drilled to accommodate almost any size Reacti-Vial Small Reaction Vial (page 54), test tube or microcentrifuge tube. These highly efficient units are constructed of an aluminum alloy for optimal thermal conductivity. To ensure proper heat transference, be sure to have a close block-to-sample container fit.

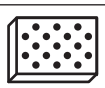
Each Reacti-Block Aluminum Block contains a thermometer well 7.1 mm dia. x 36.5 mm deep (excluding blank block J and K). Block dimensions are 9.4 cm long x 7.5 cm wide x 5.1 cm tall for all blocks except F, G, J and M which are 9.4 cm long x 7.5 cm wide x 7.6 cm tall.

The following Reacti-Block Aluminum Blocks can be used with all Reacti-Therm Modules including those equipped with Reacti-Vap Evaporators. Blocks B-1 and T-1 are specifically designed for use with Reacti-Vap Units.

**To complete your Reacti-Therm System Order:**

1. Reacti-Therm Module
2. Reacti-Block Aluminum Block(s)
3. Reacti-Therm Thermometer
4. Reacti-Vap Evaporator

### Ordering Information

| Product # | | Description |
|---|---|---|
| 18801 |  | Reacti-Block A-1<br>Holds 13 x 0.3 ml or 1 ml Reacti-Vials™; 13 holes/14 mm dia. x 23 mm deep |
| 18802 |  | Reacti-Block B-1<br>Holds 9 x 3 ml or 5 ml Reacti-Vials; 9 holes/21 mm dia. x 32 mm deep |
| 18803 |  | Reacti-Block C-1<br>Holds 13 x 3.5 ml Screw Cap Septum Vials; 13 holes/15 mm dia. x 34 mm deep |
| 18811 |  | Reacti-Block M-1<br>Holds 6 x 27.5 ml Reacti-Vials; 6 holes/28.5 mm dia. x 70 mm deep |
| 18814 |  | Reacti-Block Q-1<br>Holds 8 x 10 ml Reacti-Vials; 8 holes/26 mm dia. x 46 mm deep |
| 18816 |  | Reacti-Block S-1<br>Holds 13 x 13 mm dia. Test Tubes; 13 holes/14 mm dia. x 45 mm deep |
| 18817 |  | Reacti-Block T-1<br>Holds 9 x 16 mm dia. Test Tubes; 9 holes/17 mm dia. x 45 mm deep |
| 18818 |  | Reacti-Block U-1<br>Holds 8 x 20 mm dia. Test Tubes; 8 holes/21 mm dia. x 45 mm deep |
| 18819 |  | Reacti-Block V-1<br>Holds 17 Microcentrifuge Test Tubes; 17 holes/11 mm dia. x 45 mm deep |

## Reacti-Block Aluminum Blocks (continued)

The Reacti-Block Aluminum Blocks featured below are designed to be used exclusively with the Reacti-Therm Modules. The hole patterns do not match the needle configuration of Reacti-Vap Evaporators.

### Ordering Information

| Product # | | Description |
|---|---|---|
| 18806 |  | **Reacti-Block F**<br>Holds 8 x 5 ml Vacuum Hydrolysis Tubes; 8 holes/10 mm dia. x 64 mm deep |
| 18807 |  | **Reacti-Block G**<br>Holds 4 x 20 ml Vacuum Hydrolysis Tubes; 4 holes/19 mm dia. x 64 mm deep |
| 18809 |  | **Reacti-Block J**<br>Blank/no holes (for custom drilling) 7.6 cm tall |
| 18810 |  | **Reacti-Block K**<br>Blank/no holes (for custom drilling) 5.1 cm tall |
| 18812 |  | **Reacti-Block L**<br>Holds 16 x 0.1 ml Reacti-Vial Vials; 16 holes/12 mm dia. x 21 mm deep |

## Reacti-Vial Magnetic Stirrers

*Offer faster reaction times with smooth mixing of small samples.*

Mounted on a triangular matrix, these small Teflon-coated stirring bars fit the cone portion of 0.3, 1.0, 3.0, 5.0 and 10.0 ml Thermo Scientific Reacti-Vial Small Reaction Vials. For more information on Reacti-Vial Small Reaction Vials, see page 54.

When used with Thermo Scientific Reacti-Therm Heating/Stirring Modules, these efficient stirrers provide:

- Faster reaction times with smooth, efficient mixing of small reaction samples
- Solubilization of sticky concentrated residues such as those found on evaporation of sugar solutions
- Increased speed-of-surface reactions by keeping insoluble reactants in suspension

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 16000 | **Reacti-Vial Magnetic Stirrers**<br>For use with 3.0, 5.0 and 10 ml Reacti-Vial Small Reaction Vials | Pkg. of 6 |
| 16010 | **Reacti-Vial Magnetic Stirrers**<br>For use with 0.3 and 1.0 ml Reacti-Vial Small Reaction Vials | Pkg. of 6 |

## Hydrochloric Acid (6 N)

*Ready-to-use reagents in convenient packaging.*

Thermo Scientific Pierce Hydrochloric Acid is purified and packaged to ensure a ninhydrin negative blank on hydrolysis. Convenient, pre-scored ampule packaging of the ready-to-use HCl maintains reagent integrity. This virtually eliminates exposure to laboratory atmospheres, fingerprints and other contaminants resulting from pipetting from bulk bottles.

An excellent description of the total protein hydrolysis technique using constant boiling hydrochloric acid is detailed by Eveleigh and Winter.[1] With constant boiling hydrochloric acid, tryptophan losses are expected. Standard protein hydrolysis conditions are 105-110°C for 16-24 hours. At 150°C, this reagent can be used for the rapid (six-hour) hydrolysis of peptides.

**Reference**

1 Eveleigh, J.W. and Winter, G.D. (1970). *Protein Sequences* Ed. Needleman, S.B., Springer-Verlag, pp. 92-95.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| ✖ 24308 | **Hydrochloric Acid**<br>Constant Boiling, (6 N), Sequencing Grade | 10 x 1 ml ampules |

✖*Additional hazardous handling charge.*

## Vacuum Hydrolysis Tubes

*Completely reusable and compatible with Reacti-Therm Modules.*

Thermo Scientific Reacti-Therm Systems deliver uniform dry heat with unmatched convenience and versatility, making them an ideal choice for hydrolysis reactions.

**Applications:**

- Hydrolysis
- Sample concentration
- Lyophilization
- Hydrazinolysis

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 29550 | **Vacuum Hydrolysis Tube**<br>8 mm x 60 mm, 1 ml volume | Each |
| 29560 | **Vacuum Hydrolysis Tube**<br>10 mm x 100 mm, 5 ml volume | Each |
| 29564 | **Vacuum Hydrolysis Tube**<br>19 mm x 100 mm, 20 ml volume | Each |

## Reacti-Block Aluminum Blocks For Vacuum Reaction Tubes

*For use with Reacti-Therm Heating/Stirring Modules.*

Thermo Scientific Reacti-Therm Systems deliver uniform dry heat with unmatched convenience and versatility, making them an ideal choice for hydrolysis reactions.

### Ordering Information

| Product # | Description |
|---|---|
| 18806 | **Reacti-Block F**<br>Holds 8 x 5 ml Vacuum Hydrolysis Tubes; 8 holes/10 mm dia. x 64 mm deep |
| 18807 | **Reacti-Block G**<br>Holds 4 x 20 ml Vacuum Hydrolysis Tubes; 4 holes/19 mm dia. x 64 mm deep |
| 18809 | **Reacti-Block J**<br>Blank/no holes (for custom drilling) 7.6 cm tall |
| 18819 | **Reacti-Block V-1**<br>Holds 17 Microcentrifuge Test Tubes; 17 holes/11 mm dia. x 44 mm deep |

## Alphabetical Index

# Numerical Index

## Numerical Index

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