

Thermo Scientific Pierce Reagents, Solvents and Accessories

- Gas Chromatography
- High Performance Liquid Chromatography
- Mass Spectrometry

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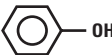
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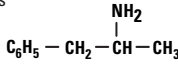
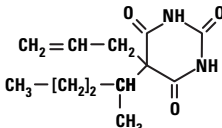
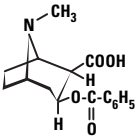
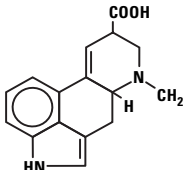
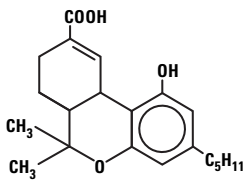
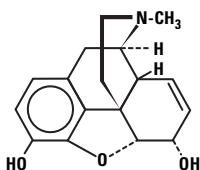
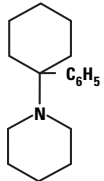
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Derivatization Reagents for Specific Functional Groups

Functional Group	Procedure	Reagent	Derivative	Notes	
<div>Amides</div> <div>$\begin{array}{c} \text{O} \\ \\ \text{--C--NH}_2 \end{array}$</div> <div>Primary</div>	Silylation	BSA	TMS Amides	Difficult to form due to steric hindrance	
		BSTFA	TMS Amides		
		BSTFA+TMCS	TMS Amides		TMCS used as a catalyst Reaction byproducts more volatile
		MSTFA	TMS Amides		
		MSTFA+TMCS	TMS Amides		
		Tri-Sil Reagents	TMS Amides		
	Acylation	MTBSTFA	TBDMCS Amides	Difficult to form; very stable	
		MTBSTFA+TBDMCS	TBDMCS Amides	TBDMCS aids derivatization	
		MBTFA	Trifluoroacetamides		
		TFAA	Trifluoroacetamides		
<div>Secondary</div> <div>$\begin{array}{c} \text{O} \\ \\ \text{--C--NHR} \end{array}$</div>	Acylation	PFAA	Pentafluoropropionamides	Good for ECD detection	
		HFBA	Heptafluorobutyamides		
	Alkylation	MethElute Reagent (TMPAH)	Methyl Amides	On-column derivatization especially for drugs	
<div>Amines</div> <div>$\begin{array}{c} \text{H} \\ \\ \text{--C--NH}_2 \\ \\ \text{H} \end{array}$</div> <div>Primary</div> <div>$\begin{array}{c} \text{H} \\ \\ \text{--C--NHR} \\ \\ \text{H} \end{array}$</div> <div>Secondary</div>	Silylation	BSA	TMS	TMCS aids derivatization	
		BSTFA	TMS		
		BSTFA+TMCS	TMS		
		MSTFA	TMS		
		MSTFA+TMCS	TMS		
		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	
<div>Carbohydrates</div> <div>$(\text{CH}_2\text{OH})_n$</div>	Silylation	MSTFA	TMS	Can be used with some syrups	
		TMSI	TMS		
		Tri-Sil Reagents	TMS		
	Acylation	MBTFA	Trifluoroacetates	Volatile derivatives of mono-, di- and trisaccharides	
TFAI	Trifluoroacetates				
<div>Carboxyl</div> <div>$\begin{array}{c} \text{O} \\ \\ \text{--C--OH} \end{array}$</div>	Silylation	BSA	TMS	Easily formed, generally not stable, analyze quickly	
		BSTFA	TMS		
		BSTFA+TMCS	TMS		
		MSTFA	TMS		
		TMCS	TMS		
		TMSI	TMS		
	Silylation	Tri-Sil Reagents	TMS	Can be used with some salts	
		MTBSTFA	TBDMCS		
		MTBSTFA+TBDMCS	TBDMCS		
	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		
<div>Hydroxyl-OH</div> <div>R--OH</div> <div>Alcohols</div> <div></div> <div>Phenols</div>	Silylation	BSA	TMS	Most often used derivatives Good thermal stability Poor hydrolytic stability Weak donor usually used with TMCS	
		BSTFA	TMS		
		BSTFA+TMCS	TMS		
		HMDS	TMS		
		MSTFA	TMS		
		MSTFA+TMCS	TMS		
		TMCS	TMS		
		TMSI	TMS		
		Tri-Sil Reagents	TMS		
		MTBSTFA	TBDMCS		
	Acylation	MTBSTFA+TBDMCS	TBDMCS	More stable than TMS, good MS fragmentation patterns TBDMCS aids derivatization	
		MBTFA	Trifluoroacetates	Good for trace analysis with EDC	
		TFAA	Trifluoroacetates	Good for trace analysis with EDC	
		TFAI	Trifluoroacetates	Good for trace analysis with EDC	
	Alkylation	PFAA	Pentafluoropropionates	Good for trace analysis with EDC	
		HFBI	Heptafluorobutrates	Good for trace analysis with EDC	
		HFAA	Heptafluorobutrates	Good for trace analysis with EDC	
PFBBr		Pentafluorobenzyl Ethers	With alkoxides only		

Derivatization Reagents for Drugs-of-Abuse

Drug	Form	Reagent	Reference
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 	Benzoyllecgonine	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

Cocaine Metabolites

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Introduction to Gas Chromatography Reagents



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. Knapp¹, 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 analysis⁴
2. To improve the analytical efficiency of the compound^{5,6}
3. To improve the detectability of the compound⁷

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 prior to 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 (TR-WAX or TR-WaxMS) and free fatty acid phases (TR-FFAP).

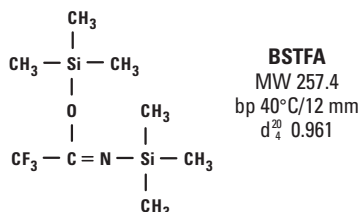
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BSTFA

For excellent chromatographic separations.



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(CH₃)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.

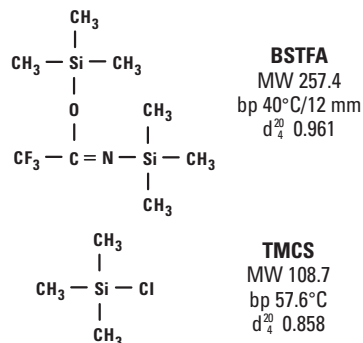
NOTE: This protocol is not recommended for sugars.

Ordering Information

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

BSTFA + TMCS

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



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

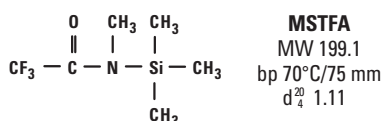
1. Combine 5-10 mg sample, 0.5 ml Pierce BSTFA + 1% TMCS and 1.0 ml solvent (acetonitrile is recommended for amino acids) in a 3.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Cap vial and shake for 30 seconds.
3. Heat at 7°C for 15 minutes.
4. Analyze by gas chromatography.

Ordering Information

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

MSTFA and MSTFA 1% TMCS

Offers maximum volatility.



Highlights:

- Trimethylsilyl donor strength comparable to BSA and BSTFA
- Reacts to replace labile hydrogens on a wide range of polar compounds with a $-\text{Si}(\text{CH}_3)_3$ 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. 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. 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.

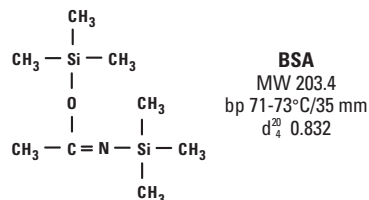
Ordering Information

Product #	Description	Pkg. Size
✖ TS-48910	MSTFA (N-Methyl-N-trimethylsilyltrifluoroacetamide)	10 x 1 ml ampules
✖ TS-48911	MSTFA	10 g Hypo-Vial Sample Storage Vial
✖ TS-48913	MSTFA	25 ml Hypo-Vial Sample Storage Vial
✖ TS-48914	MSTFA	100 ml Hypo-Vial Sample Storage Vial
✖ TS-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.



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 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 poly-hydroxyl 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.

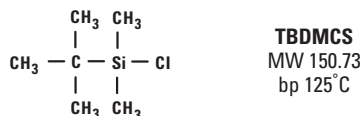
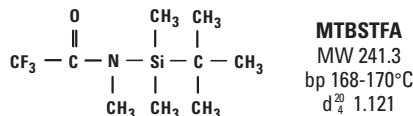
NOTE: Material is silylated at room temperature within times varying from a few minutes to a few hours. Heating will hasten reaction.

Ordering Information

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

MTBSTFA and MTBSTFA + 1% TBDMCS

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



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
- 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.

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.)

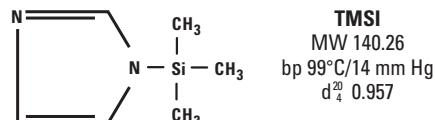
Ordering Information

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

✖ Additional hazardous handling charge.

TMSI

The strongest hydroxyl silylator available for carbohydrates and steroids.



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

Thermo Scientific Pierce TMSI is unique, as it reacts quickly and smoothly with hydroxyls and carboxylic acids, but not with amines. 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).

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.

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. 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 HFBT, TEAL 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.

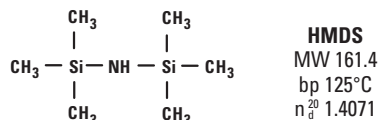
Ordering Information

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

✖ Additional hazardous handling charge.

HMDS

The popular choice for silylation of sugars and related substances.



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.*

PROTOCOL 1

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

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.

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.

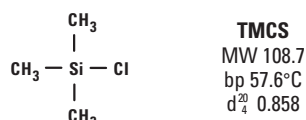
Ordering Information

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

✖ Additional hazardous handling charge.

TMCS

An excellent catalyst for difficult-to-silylate compounds.



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

PROTOCOL

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

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.

Ordering Information

Product #	Description	Pkg. Size
✖ TS-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.*

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.

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% NaHCO₃ 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.

Ordering Information

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

✖ Additional hazardous handling charge.

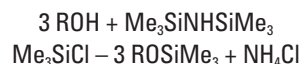
Tri-Sil HTP (HMDS:TMCS:pyridine) Reagent

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
- Alcohols
- Phenols
- Steroids
- Sterols
- Bile acids and other organic acids
- Some amines

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



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.

Ordering Information

Product #	Description	Pkg. Size
✖ TS-48999	Tri-Sil HTP Reagent HMDS:TMCS:Pyridine (2:1:10)	10 x 1 ml ampules
✖ TS-49001	Tri-Sil HTP Reagent HMDS:TMCS: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.

Ordering Information

Product #	Description	Pkg. Size
✖ TS-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:TMCS)

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.

Ordering Information

Product #	Description	Pkg. Size
✖ TS-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 reacts 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.

Ordering Information

Product #	Description	Pkg. Size
✗ TS-49230	Tri-Sil TP Reagent TMSI: Pyridine (1:4)	10 x 1 ml ampules
✗ TS-49231	Tri-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:

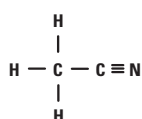
- Purified, dried and packaged under nitrogen in convenient Hypo-Vial Sample Storage Vials
- Supplied with elastomer septa, allowing immediate access to sample without exposure to moisture and oxygen
- Use polar solvents (acetonitrile, dimethylformamide, dimethylsulfoxide, pyridine and tetrahydrofuran) 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

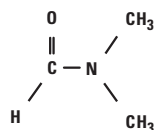
Product #	Description	Pkg. Size
✗ TS-20062	Acetonitrile	50 ml Hypo-Vial Sample Storage Vial
✗ TS-20672	Dimethylformamide	50 ml Hypo-Vial Sample Storage Vial
TS-20684	Dimethylsulfoxide	50 ml Hypo-Vial Sample Storage Vial
✗ TS-27530	Pyridine	50 ml Hypo-Vial Sample Storage Vial
✗ TS-27860	Tetrahydrofuran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

✗ Additional hazardous handling charge.

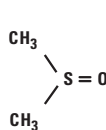
For HPLC Grade Solvents, see page 27, 41.



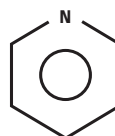
Acetonitrile
MW 41.05
bp 81.6°C



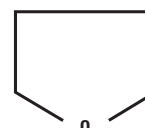
Dimethylformamide
MW 73.09
bp 153°C



Dimethylsulfoxide
MW 78.13
bp 189°C



Pyridine
MW 79.10
bp 115.2°C



Tetrahydrofuran
MW 72.10
bp 66°C

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 chromatography² 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.

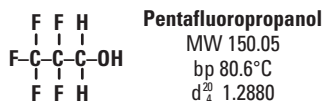
Acyl derivatives also are useful in MS applications in which they direct the fragmentation patterns of the compounds to be studied.⁴

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 heptafluorobutyl 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.



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
TS-65195	Pentafluoropropanol	10 x 1 ml ampules

Thermo Scientific TRACE GC Capillary Columns

The heart of any gas chromatograph is the column. We have used the knowledge gained over 30 years of HPLC column production and 50 years in GC and GC/MS instrument manufacturing to offer a range of columns and consumables that are unsurpassed in the most important aspects of their performance.

A leap forward in column performance

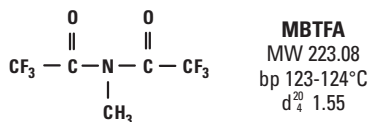
- Low bleed even at elevated temperatures
- Outstanding robustness for difficult separations
- Reliable and reproducible performance
- Complete range of GC consumables for all instruments

www.thermo.com/columns



MBTFA

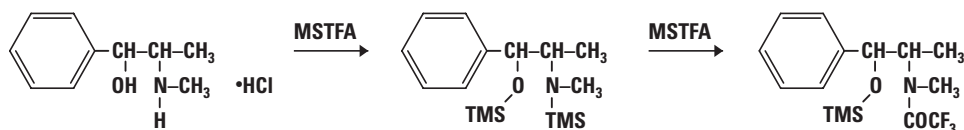
For trifluoroacetylating primary and secondary amines, hydroxyl and thiol groups, and carbohydrates.



Thermo Scientific Pierce MBTFA is ideal for trifluoroacetylating primary and secondary amines, hydroxyl and thiol groups, or carbohydrates under nonacidic conditions. 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.

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



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.

PROTOCOL 1

For trifluoroacetylating 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 trifluoroacetylating sugars.

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.

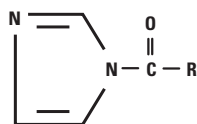
Ordering Information

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

✖ Additional hazardous handling charge.

Perfluoroacylimidazoles (TFAI and HFBI)

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 ₃ F 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 alkylamines to quantitatively derivatize the indole nitrogen, as well as other functional groups present. 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.

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.

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.

Ordering Information

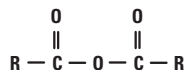
Product #	Description	Pkg. Size
✖ TS-48882	TFAI (Trifluoroacetylimidazole)	10 x 1 ml ampules
✖ TS-44211	HFBI (Heptafluorobutyrylimidazole)	5 g bottle

✖ 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 ₂ F ₅	PFAA	310.0	74°C	1.571
C ₃ F 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.

Ordering Information

Product #	Description	Pkg. Size
✗ TS-67363	TFAA (Trifluoroacetic Acid Anhydride)	100 g bottle
✗ TS-65193	PFAA (Pentafluoropropionic Acid Anhydride)	10 x 1 ml ampules
✗ TS-65192	PFAA	25 g bottle
✗ TS-65191	PFAA	100 g bottle
✗ TS-63164	HFAA	10 x 1 ml ampules
✗ TS-63163	HFAA	25 g bottle
✗ TS-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-aromatic¹ (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 acids into esters, which produce better chromatograms than the free acids.

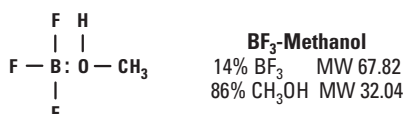
In addition, alkylation reactions can be used to prepare ethers, thioethers and thioesters; *N*-alkylamines; and amides.² As the acidity of the active hydrogen decreases, the strength of the alkylating reagent must be increased. As the reagents and conditions become harsher, the selectivity and applicability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

BF₃-Methanol

Provides convenient, fast and quantitative esterification of fatty acids.



BF₃-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 BF₃-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 BF₃-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 BF₃-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 Na₂SO₄.
7. Evaporate solvent under nitrogen.
8. Analyze by gas chromatography.

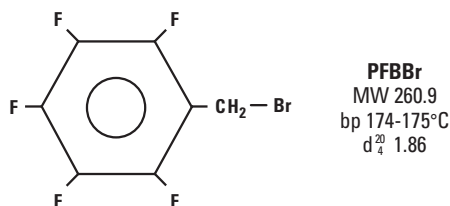
Ordering Information

Product #	Description	Pkg. Size
✖ TS-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.



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.

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. 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.

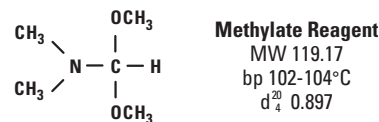
Ordering Information

Product #	Description	Pkg. Size
✗ TS-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.



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 acids¹ 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.

Ordering Information

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

MethElute Reagent (TMPAH)

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

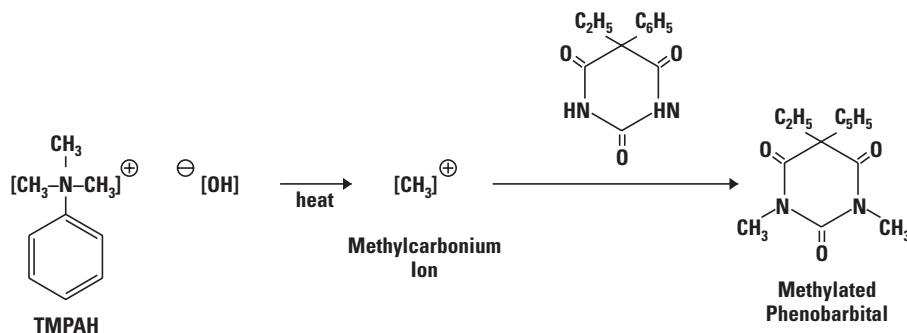


Figure 1. Thermo Scientific Pierce MethElute Reagent reaction with phenobarbital.

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, sedatives, xanthine bases, phenolic alkaloids and Dilantin 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. 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.

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

Sensitivity. Detects barbiturates down to 0.2 mg/dl.

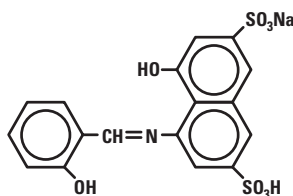
Ordering Information

Product #	Description	Pkg. Size
✖ TS-49300	MethElute Reagent (TMPAH)	10 ml Hypo-Vial Sample Storage Vial
✖ TS-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.* 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.

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. 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.

Ordering Information

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



Thermo Scientific Pierce Siliconizing Fluids

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:

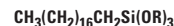
- For clean drainage and elimination of meniscus
- To reduce adsorption of polar compounds, proteins and trace metals onto glass surfaces and reduce leaching of trace metals into solution
- To prevent current tracking and minimize electrical leakage on glass surfaces and ceramics
- To protect delicate 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.



Pierce Siliconizing Fluid – Water Soluble

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 Siliconizing 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.

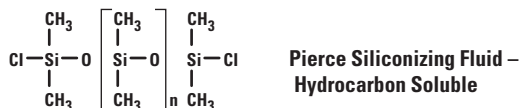
Ordering Information

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

✖ Additional hazardous handling charge.

Pierce Siliconizing Fluid – Hydrocarbon Soluble

A hydrocarbon soluble fluid for siliconizing glass surfaces.



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 Siliconizing 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

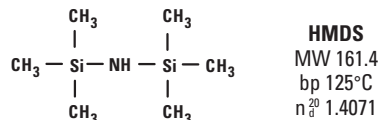
Ordering Information

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

✗ Additional hazardous handling charge.

Monofunctional Silane – HMDS

Ideal for active surface site deactivation.



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.

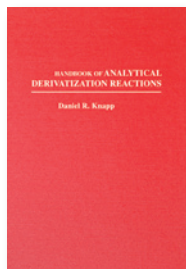
Ordering Information

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

TS-15012	Handbook of Analytical Derivatization Reactions Knapp, D.R. Ed (1979) Published by John Wiley and Sons, Inc. Hardcover, 741 pages
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Introduction to 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
✗ TS-25003	Heptafluorobutyric Acid, Sequencing Grade	100 ml
✗ TS-53104	Heptafluorobutyric Acid, HPLC Grade	10 x 1 ml

✗ Additional hazardous handling charge.

Thermo Scientific Binding and Elution Buffers for Affinity Purification

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

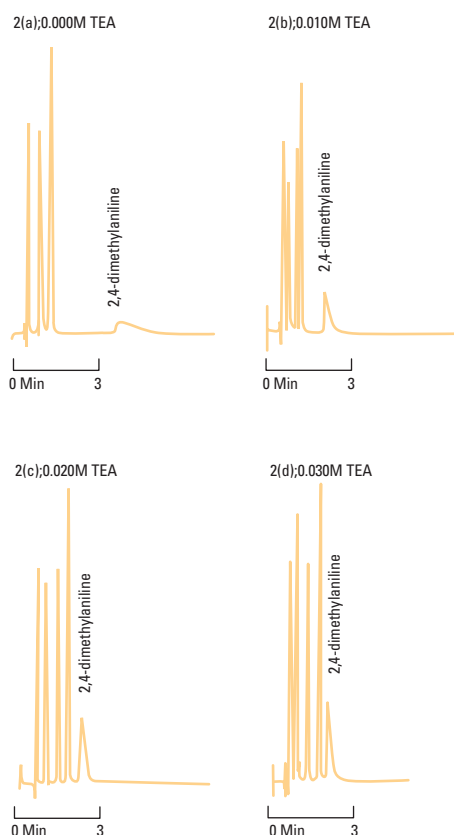


Figure 1A-1D. Effect of TEA concentration on a mixture of basic antihistamines and 2,4-dimethylaniline* 150 mm x 4.6mm C8. Conditions: a) 40% methanol, 0.060 M HSA sodium salt, 0.045 M citric acid; b) 0.150 M citric acid, 0.060 M TEA, pH 7.5 with NaOH; c) 0.150 M citric acid, pH 7.5 with NaOH; isocratic with TEA concentrations modified by varying b/c ratio, 3 min./ml, 50°C, 254 nm.

Ordering Information

Product #	Description	Pkg. Size
✖ TS-53101	Triethylamine (TEA), HPLC Grade	25 g
✖ TS-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
✖ TS-28905	Formic Acid 99+%	10 x 1 ml ampules

✖ Additional hazardous handling charge.

Thermo Scientific Pierce HPLC Ion Pair Reagents

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 HPLC¹⁻³
- Protein/peptide sequencing⁴⁻⁷
- Protein/peptide solubilizing agent⁴⁻⁷
- Solid-phase peptide synthesis⁸
- 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).

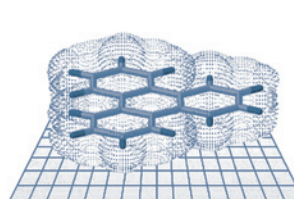
Because the density of trifluoroacetic acid is 1.53 g/ml the difference between 0.1% TFA (w/v) and 0.1% TFA (v/v) is more than 50%. For the sake of reproducibility, it is essential for authors of a method to specify, and for users of a method to know, whether the TFA concentration is given as “w/v” or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

Thermo Scientific Hypercarb Columns

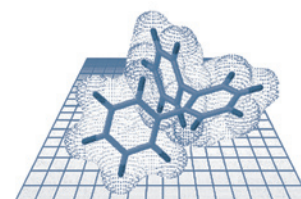
100% porous graphitic carbon for extended separation capabilities

- Exceptional retention of very polar analytes
- Separates structurally related substances
- pH stable from 0 to 14
- Ideal for high temperature applications

www.thermo.com/columns

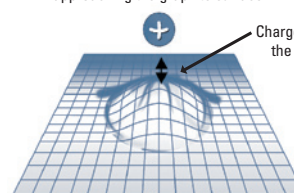


A planar compound can align itself closely with the Hypercarb® surface resulting in more interaction and retention.



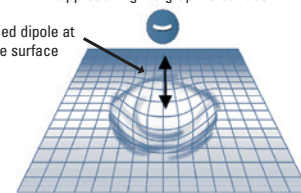
A non-planar molecule cannot align itself as closely with the Hypercarb surface resulting in less interaction and retention.

Electrostatic charge of polar analyte approaching the graphite surface



Electrons in the polarizable surface of the graphite attracted towards positive charge

Electrostatic charge of polar analyte approaching the graphite surface



Electrons in the polarizable surface of the graphite repelled away from a negative charge

References

1. Chic, R.M. and Regnier, F.E. (1990) *Methods Enzymol.* **182**, 392-421.
2. Zarembek, 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 Edition. 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
✖ TS-28901	Trifluoroacetic Acid, Sequencing Grade	500 ml
✖ TS-28902	Trifluoroacetic Acid, Sequencing Grade	10 x 1 g
✖ TS-28903	Trifluoroacetic Acid, Sequencing Grade	100 g
✖ TS-28904	Trifluoroacetic Acid, Sequencing Grade	10 x 1 ml ampules

✖ Additional hazardous handling charge.

Thermo Scientific HPLC/Spectrophotometric Grade Solvents

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 (CH₃)₂
- Purity (GC): ≥99%
- MW: 73.09
- Density: 0.944
- B.P. 153°C
- Water: <0.1%

Dimethylsulfoxide (DMSO), Sequencing Grade

- C₂H₆OS
- Purity (GC): >99.5%
- MW: 78.13
- Density: 1.101
- Water: ≤0.2%

Pyridine

- C₅H₅N
- Purity (GC): ≥99%
- MW: 79.10
- Density: 0.978
- B.P.: 115°C

Ordering Information

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

✖ Additional hazardous handling charge.
For GC Grade Solvents, see page 12.

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
TS-31700	Peptide Retention Standard, S1-S5 Contains: 5 C-terminal amide decapeptides, 4 of which are N-acetylated with the sequence variation as follows: AC-Arg-Gly-X-X-Gly-Leu-Gly-Leu-Gly-Lys-Amide; Gly ² -Gly ⁴ -Ala ³ -Gly ¹ , Val ³ -Gly ⁴ and Val ³ -Val ⁴ . The fifth peptide, Ala ³ -Gly ⁴ , contains a free N-amino group. This mixture will provide 100-200 injections at 0.1 AUFS at 210 nm.	1 vial

Thermo Scientific Pierce Derivatization and Visualization Reagents for HPLC

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 2). 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.

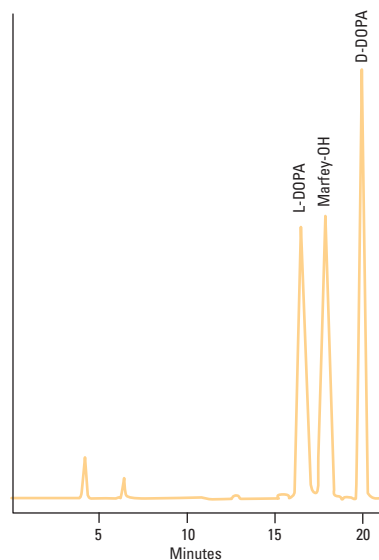


Figure 1. Separation of D- and L-DOPA on 100 mm x 4.6 mm C18.

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 $\sim 3 \times 10^4$ and can be detected by UV at 340 nm with picomole sensitivity.

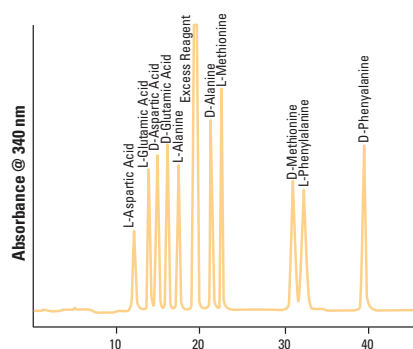
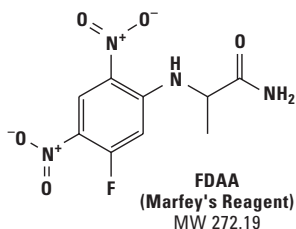


Figure 2. Separation of D- and L-amino acids on 100 mm x 4.6 mm C18.
Conditions: A) 0.05 M triethylamine phosphate, pH 3.0;
B) acetonitrile. Linear gradient: 10 to 40% B in 45 minutes, 2.0 ml/minute, 25°C, 340 nm.



PROTOCOL

Preparation of FDAA Derivatives

1. Place 100 μ l (5 μ mol) sample in a 1.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Add 200 μ l of a 1% (w/v) solution of Pierce FDAA in acetone. Add 40 μ l of 1.0 M sodium bicarbonate. μ mol FDAA: μ mol amino acid should be 1.5:1.0.
3. Heat at 40°C for 1 hour. Remove and cool.
4. Add 20 μ l 2 M HCl. Allow sample to degas.
5. Analyze. Conditions: 100 mm x 4.6 mm C18; 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

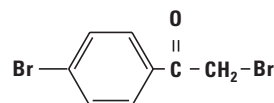
1. Marfey, P. (1984). *Carlsberg Res. Comm.* **49**, 591-596.

Ordering Information

Product #	Description	Pkg. Size
TS-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 μ mol/ml p-Bromophenacylbromide, 0.005 μ mol/ml crown ether in acetonitrile)

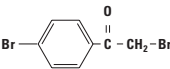
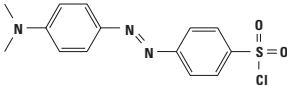
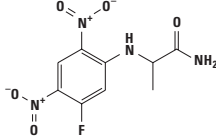
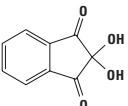
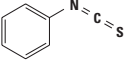
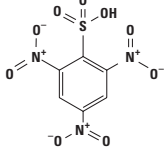
1. Dissolve \sim 10 mg acid in MeOH in a 5.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial fitted with Thermo Scientific Reacti-Vial Magnetic Stirrer. Neutralize to the phenolphthalein endpoint with KOH/MeOH.*
2. Evaporate the MeOH with N₂.
3. Add 1.0 ml Phenacylate Reagent and 2.0 ml dry CH₃CN.
4. Heat at 80°C with stirring for 30 minutes.
5. Remove and cool.
6. Analyze. Conditions: C18; UV at 250 nm
A: CH₃CN; B: deionized H₂O
Linear gradient: 80% A to 100% A; Flow: 2.0 ml/minute

* If the formation of potassium salts is undesirable, neutralize by adding KHCO₃ at five times the total acid instead of using KOH.

Ordering Information

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

Thermo Scientific Pierce Detection Reagents for HPLC

Functional Group	Description	Detection*	Page	Comments
Carboxylic Acid $\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{C}-\text{OH} \end{array}$	<i>p</i>-Bromophenacylate 	UV	29	Formulation of 1.0 mmol/ml <i>p</i> -bromophenacyl bromide and 0.005 mmol/ml crown ether in acetonitrile; pre-column; nanomole detection levels: $\lambda_{\text{max}} = 260 \text{ nm}^{1-7}$
Primary Amine $\begin{array}{c} \text{R}-\text{N}-\text{H} \\ \\ \text{H} \end{array}$	Dabsyl Chloride 	Vis	40	4-N, N-dimethylaminoazobenzene-4'-sulfonyl chloride (dabsyl chloride); pre-column; nanomole detection levels: $\lambda_{\text{max}} = 436 \text{ nm}^{8-14}$
	FDAA, Marfey's Reagent 	UV	29, 40	1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA); pre-column; nanomole detection levels: $\lambda_{\text{max}} = 340 \text{ nm}$. For chiral separations of amino acids. ^{15, 28-29}
	Ninhydrin 	Vis	38	Post-column; nanomole detection levels: $\lambda_{\text{max}} = 570 \text{ nm}^{22}$
	PITC 	UV	39	Phenylisothiocyanate (PITC); pre-column; picomole detection levels: $\lambda_{\text{max}} = 254 \text{ nm}^{23-24}$
	TNBSA  TNBSA MW 293.17	EC, UV	36	2,4,6-Trinitrobenzene-sulfonic acid (TNBSA); pre- or post-column; nanomole detection levels with EC and UV, GC - 0.85V; $\lambda_{\text{max}} = 250 \text{ nm}^{25-26}$
Secondary Amine $\text{R}-\text{NH}-\text{R}$	Ninhydrin (see structure above)	Vis	38	Post-column; nanomole detection levels: $\lambda_{\text{max}} = 440 \text{ nm}^{22}$
	PITC (see structure above)	UV	39	Phenylisothiocyanate (PITC); pre-column; picomole detection levels: $\lambda_{\text{max}} = 254 \text{ nm}^{23-24}$

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

Detection Reagents

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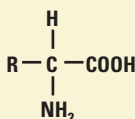
Introduction to Amino Acid Analysis



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 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.

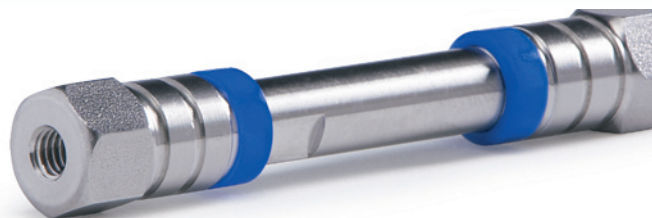
Two of the most widely used of these methods involve the formation of dansyl¹⁸⁻¹⁹ or *o*-phthalaldehyde (OPA)²⁰⁻²⁴ derivatives of amino acids prior to HPLC analysis. Both methods offer greater sensitivity and shorter analysis time than post-column derivatization techniques. Other methods include the 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.

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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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Thermo Scientific Pierce Hydrolysis Reagents

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

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Ordering Information

Product #	Description	Pkg. Size
✖ TS-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 μ moles/ml in 0.1 N HCl. The following amino acids are included with our Amino Acid Standard H:

- L-Alanine
- Ammonia [(NH₄)₂ SO₄]
- 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.

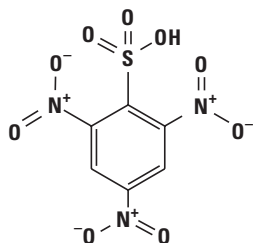
Ordering Information

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

Thermo Scientific Pierce Fluorometric Detection Reagents for Amino Acids

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 solution at pH 8 to form yellow adducts. No colored derivatives are formed with the secondary amino acids proline and hydroxyproline. The colored derivatives are monitored at 345 nm and have extinction coefficients in the range of $1\text{--}1.5 \times 10^4$.

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 ($\lambda_{\text{max}} = 335 \text{ nm}$)

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Ordering Information

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

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Thermo Scientific Pierce

Ninhydrin Detection Reagents 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.

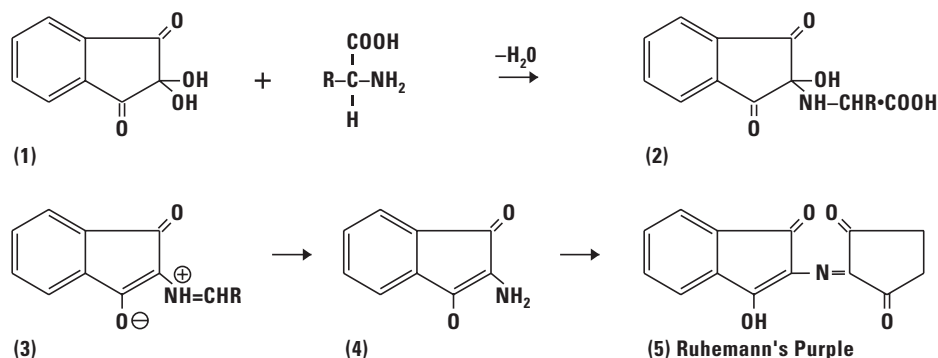
The color reaction between amino-containing compounds and ninhydrin (2,2-dihydroxy-1,3-indandione) is very sensitive. McCaldin has studied all phases of ninhydrin chemistry and proposed a mechanism for the reaction of ninhydrin with amino acids, accounting for the aldehydes, carbon dioxide, ammonia and 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 5.20¹⁰ 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.

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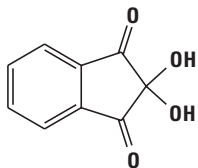


Reaction Scheme. The course of the ninhydrin reaction with amino acids is as follows:

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
TS-21003	Ninhydrin	500 g

Thermo Scientific Premium Vials

Compatible with a wide range of HPLC and GC instruments

Thermo Scientific premium vials are compatible with any manufacturers instruments. Made from highly pure borosilicate glass, our vials meet the high standards required by such bodies as the US and European Pharmacopoeia, and ASTM for hydrolytic extraction.

- Convenient clam shell packs include 100 vials and caps
- Vials and caps are also available separately
- Head space vials also available

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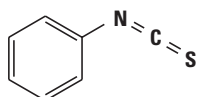


Thermo Scientific Pierce

High-Purity Pre-Column Derivatization Reagents

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: H₂O, 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.
3. Janssen, *et al.* (1986). *Chromatogr.* **22**(7-12).
4. Evert, R.F. (1986). *Anal. Biochem.* **154**, 431-435.

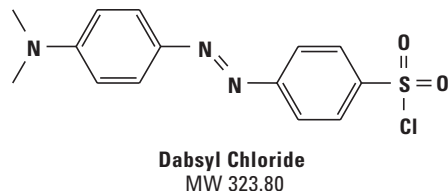
Ordering Information

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

✗ Additional hazardous handling charge.

Dabsyl Chloride

It's recrystallized twice for twice the quality!



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 polypeptides¹
- Analysis of phospho-amino and amino acid amides³
- Analysis of amino acid neurotransmitters in mouse brain⁴
- Optimal reaction conditions⁵

References

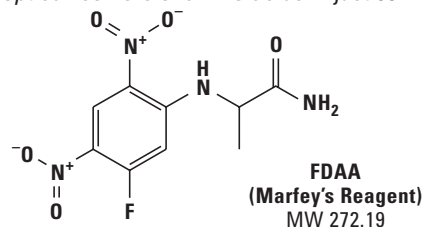
1. Chang, J.Y., *et al.* (1981). *Biochem. J.* **199**, 547-555.
2. Chang, J.Y., *et al.* (1982). *Biochem. J.* **199**, 803-806
3. Chang, J.Y. (1984). *J. Chromatogr.* **295**, 193-200.
4. Chang, J.Y., *et al.* (1981). *FEBS Lett.* **132**, 117-120.
5. Vendrell, J., *et al.* (1986). *J. Chromatogr.* **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
TS-21720	Dabsyl Chloride (4-Dimethylaminoazobenzene-4-Sulfonyl Chloride)	500 mg

FDAA, Marfey's Reagent

Derivatizes optical isomers of amino acids in just 90 minutes.



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 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 3×10^4 . 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 μ l (5 μ moles) sample in a 1.0 ml Thermo Scientific Reacti-Vial Small Reaction Vial.
2. Add 200 μ l of 1% (w/v) solution of Pierce FDAA in acetone. Add 40 μ l of 1.0 M sodium bicarbonate (μ moles FDAA: μ moles amino acid should be 1.5:1.0.)
3. Heat at 40°C for 1 hour. Remove and cool.
4. Add 20 μ l 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: CH₃CN
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
TS-48895	FDAA Marfey's Reagent (1-fluoro-2, 4-dinitrophenyl-5-L-alanine amide)	50 mg

Thermo Scientific Pierce Solvents for Amino Acid Analysis

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

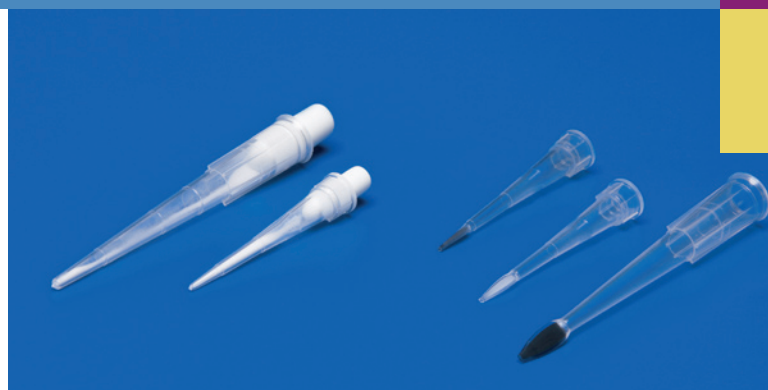
- HCON (CH₃)₂
- Purity (GC): ≥99%
- MW: 73.09
- Density: 0.944
- B.P. 153°C
- Water: <0.1%

Dimethylsulfoxide (DMSO), Sequencing Grade

- C₂H₆OS
- Purity (GC): >99.5%
- MW: 78.13
- Density: 1.101
- Water: ≤0.2%

Pyridine

- C₅H₅N
- Purity (GC): ≥99%
- MW: 79.10
- Density: 0.978
- B.P.: 115°C



Thermo Scientific HyperSep Tip Microscale Solid Phase Extraction

Revolutionary micro pipette tip for sample preparation

- Revolutionary innovative SPE cartridge design
- Faster sample preparation
- Minimal sample loss
- No contamination from a supporting matrix
- Separation in volumes as low as 100 nanoliters

Thermo Scientific HyperSep SpinTip Microscale Solid Phase Extraction

A unique concept in solid phase extraction

- Faster sample preparation with minimal sample loss
- No contamination from the supporting matrix
- Samples volumes as small as 0.1 µL can be handled
- Available in volumes of 1- 10 µL and 10- 200 µL

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Ordering Information

Product #	Description	Pkg. Size
✗ TS-51101	Acetonitrile	1 L
TS-51140	Water	1 L
✗ TS-20673	Dimethylformamide (DMF)	50 ml
✗ TS-25104	Pyridine (C ₅ H ₅ N)	100 g
TS-20688	Dimethylsulfoxide (DMSO)	950 ml

✗ Additional hazardous handling charge.

For GC Grade DMF and DMSO, see page 12.

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 be 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).

Thermo Scientific Vials and Closures

Reacti-Vial Small Reaction Vials

Size	Dimensions (Diam. x Height) (mm ± 1mm)	Inside Diameter (mm)	Thread Style	Clear Pkg. of 12	Amber Pkg. of 12
				Product #	Product #
100 µl	12 x 32	8	425-8	TS-13100	
0.3 ml	13 x 32	11	425-13	TS-13220	
1.0 ml	13 x 45	11	425-13	TS-13221	TS-13097
3.0 ml	20 x 47	18	425-20	TS-13222	
5.0 ml	20 x 60	18	425-20	TS-13223	TS-13099
10.0 ml	25 x 69	22	425-24	TS-13225	

Optional Accessories

Vial Size	Teflon/ Silicone Discs Pkg. of 72	Rubber Laminated Discs Pkg. of 72	Open-Top Screw Caps Pkg. of 72	Miniert Valves Pkg. of 72	Reacti-Vial Magnetic Stirrers Pkg. of 6
	Product #	Product #	Product #	Product #	Product #
100 µl	TS-12708		TS-13208		
0.3 ml	TS-12712	TS-12412	TS-13215		TS-16010
1.0 ml	TS-12712	TS-12412	TS-13215		TS-16010
3.0 ml	TS-12718	TS-12418	TS-13218	TS-10135	TS-16000
5.0 ml	TS-12718	TS-12418	TS-13218	TS-10135	TS-16000
10.0 ml	TS-12722	TS-12422	TS-13219	TS-10130	TS-16000

*For more information about discs and Mininert™ Valves, see page 46, 47.

*For more information about Reacti-Vial Magnetic Stirrers, see page 51.

Thermo Scientific HyperSep Retain Polymeric SPE

Fast & Easy SPE Method Development

- HyperSep™ Retain™ PEP for polar and non-polar analyte retention
- HyperSep Retain-CX for enhanced retention of basic compounds
- HyperSep Retain-AX for enhanced retention of acidic compounds
- High capacity materials
- pH stable 0-14

HyperSep Retain polymeric materials have been designed to give balanced retention of a range of analyte types. HyperSep Retain PEP is a high purity polystyrene DVB material which has been modified with hydrophilic groups to give retention of both polar and non-polar analytes. The problems associated with column drying in traditional silica-based SPE materials are eliminated when using this product range.

Application Areas:

- Drugs and metabolites in biological fluids
- Peptides in serum, plasma or biological fluids

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Discs and Septa for Hypo-Vial Storage Vials

A variety of choices to fit your exact needs.

Ordering Information

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

*For disc and septum chemical compatibility guide, see page 47.

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
TS-13504	Pre-Cleaned Vials	3.5 ml, clear, pkg. of 72
TS-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
TS-12995	Hypo-Vial Sample Storage Vials	125.0 ml, clear, Pkg. of 84
TS-13214	Aluminium Crimp Seals for Hypo-Vial Storage Vials	Pkg. of 144

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
TS-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	Amber Pkg. of 12
				Product #	Product #
1.5 ml	12 x 32	8	425-8		TS-13080
3.5 ml	15 x 45	12	425-13	TS-13019	
7.0 ml	17 x 60	13	425-15	TS-13028	
14.0 ml	21 x 70	16	425-18	TS-13043	
40.0 ml	27 x 95	22	425-24	TS-13075	

Optional Accessories

Vial Size	Teflon/ Silicone Discs Pkg. of 72	Teflon/ Rubber Laminated Discs Pkg. of 72	Mininert Valves Pkg. of 72	Open-Top Screw Caps Pkg. of 72
	Product #	Product #	Product #	Product #
1.5 ml	TS-12708			TS-13208
3.5 ml	TS-12712	TS-12412		TS-13215
7.0 ml	TS-12713			TS-13216
14.0 ml	TS-12716			TS-13217
40.0 ml	TS-12722	TS-12422	TS-10130	TS-13218

For disc and discs and Mininert Valves, see page 46, 47.

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:

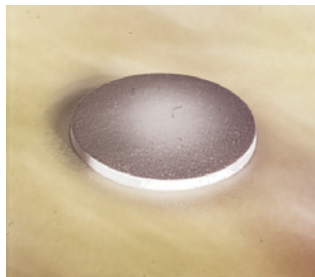
- Reseals instantly puncture after puncture
- Compresses, giving it a “lock-washer” effect in maintaining a tight seal, and forces the Teflon coating to conform to the sealing surface
- No bent needles from a septum that is too hard; standard syringe and CC needles penetrate the entire disc with ease
- 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
TS-12708	75	10	100 µl Reacti-Vial Small Reaction Vials 1.5 ml Screw Cap Septum Vials
TS-12712	90	10	0.3 and 1.0 ml Reacti-Vial Small Reaction Vials 3.5 ml Screw Cap Septum Vials
TS-12713	90	10	7 ml Screw Cap Septum Vials
TS-12716	90	10	14 ml Screw Cap Septum Vials
TS-12718	90	10	3 and 5 ml Reacti-Vial Small Reaction Vials
TS-12720	125	10	6-125 ml Hypo-Vial Sample Storage Vials
TS-12722	90	10	25 and 40 ml Screw Cap Septum Vials 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
TS-12412	12 mm	0.3 and 1.0 ml Reacti-Vial Small Reaction Vials, 3.5 ml Screw Cap Septum Vials
TS-12418	18 mm	3 and 5 ml Reacti-Vial Small Reaction Vials
TS-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
TS-10135	Mininert Valves	3 and 5 ml Reacti-Vial Small Reaction Vials	20 mm	12/pkg
TS-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	Trifluoroacetic 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

Thermo Scientific Products for Heating/Stirring/Evaporation



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 50-51). 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

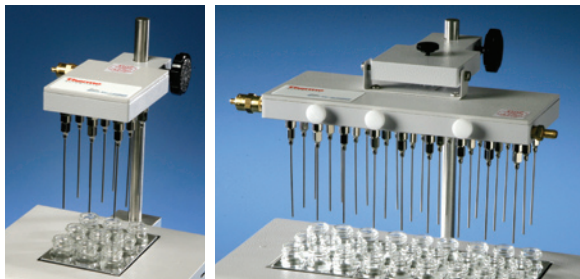
Product #	Description	Pkg. Size
TS-18820	Reacti-Probe Remote Temperature Probe	1 unit
TS-18821	Reacti-Therm Heating/Stirring Module (Single Block)	1 unit
TS-18822	Reacti-Therm Heating Module (Single Block)	1 unit
TS-18823	Reacti-Therm Heating/Stirring Module (Triple Block)	1 unit
TS-18824	Reacti-Therm III Heating Module (Triple Block)	1 unit
TS-18825	Reacti-Vap Evaporator	1 unit
TS-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
TS-18825	Reacti-Vap Evaporator (9-port) For use with Reacti-Therm Single Block Modules; 18822 and 18821, Available Fall 2008 Includes 9 needles and plugs
TS-18826	Reacti-Vap III Evaporator (27-port) For use with Reacti-Therm III Modules; 18823 and 18824, Available Fall 2008 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
TS-18782	Reacti-Vap Replacement Tube Kit 2.5 inch (64 mm)	Pkg. of 9 and plugs
TS-18784	Reacti-Vap Teflon Coated Needles 4-inch (102 mm) x 19 gauge	Pkg. of 9
TS-18786	Reacti-Vap Teflon Coated Needles 6-inch (152 mm) x 19 gauge	Pkg. of 9

Reacti-Therm Thermometers

Teflon-coated, designed specifically for dry incubations.

Ordering Information

Product #	Description
TS-18914	Reacti-Therm Thermometer, Mercury-free (0-100°C)
TS-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 42), 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
TS-18801		Reacti-Block A-1 Holds 13 x 0.3 ml or 1 ml Reacti-Vials™; 13 holes/14 mm dia. x 23 mm deep
TS-18802		Reacti-Block B-1 Holds 9 x 3 ml or 5 ml Reacti-Vials; 9 holes/21 mm dia. x 32 mm deep
TS-18803		Reacti-Block C-1 Holds 13 x 3.5 ml Screw Cap Septum Vials; 13 holes/15 mm dia. x 34 mm deep
TS-18811		Reacti-Block M-1 Holds 6 x 27.5 ml Reacti-Vials; 6 holes/28.5 mm dia. x 70 mm deep
TS-18814		Reacti-Block Q-1 Holds 8 x 10 ml Reacti-Vials; 8 holes/26 mm dia. x 46 mm deep
TS-18816		Reacti-Block S-1 Holds 13 x 13 mm dia. Test Tubes; 13 holes/14 mm dia. x 45 mm deep
TS-18817		Reacti-Block T-1 Holds 9 x 16 mm dia. Test Tubes; 9 holes/17 mm dia. x 45 mm deep
TS-18818		Reacti-Block U-1 Holds 8 x 20 mm dia. Test Tubes; 8 holes/21 mm dia. x 45 mm deep
TS-18819		Reacti-Block V-1 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
TS-18806		Reacti-Block F Holds 8 x 5 ml Vacuum Hydrolysis Tubes; 8 holes/10 mm dia. x 64 mm deep
TS-18807		Reacti-Block G Holds 4 x 20 ml Vacuum Hydrolysis Tubes; 4 holes/19 mm dia. x 64 mm deep
TS-18809		Reacti-Block J Blank/no holes (for custom drilling) 7.6 cm tall
TS-18810		Reacti-Block K Blank/no holes (for custom drilling) 5.1 cm tall
TS-18812		Reacti-Block L 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 42.

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
TS-16000	Reacti-Vial Magnetic Stirrers For use with 3.0, 5.0 and 10 ml Reacti-Vial Small Reaction Vials	Pkg. of 6
TS-16010	Reacti-Vial Magnetic Stirrers For use with 0.3 and 1.0 ml Reacti-Vial Small Reaction Vials	Pkg. of 6

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