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.1-3

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,2,4 to isolate and purify derivatives by extraction in an organic solvent, followed by washing with aqueous solutions.2,5 Reagents that introduce a t-butyldimethylsilyl group instead of the trimethylsilyl group were developed for greater hydrolytic stability.5 These derivatives provide improved stability against hydrolysis and provide distinctive fragmentation patterns, making them useful in GC/MS applications.5

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).

References
**BSTFA**

For excellent chromatographic separations.

\[
\begin{align*}
\text{CH}_3 & \quad \text{BSTFA} \\
\text{CH}_3 & \quad \text{MW 257.4} \\
\text{O} & \quad \text{bp 40°C/12 mm} \\
\text{CF}_3 & \quad \text{d}^\circ 0.961 \\
\end{align*}
\]

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 \((N,O-\text{Bis(trimethylsilyl)}\) acetamide). BSTFA reacts to replace labile hydrogens on a wide range of polar compounds with a \(-\text{Si(CH}_3)_3\) group. This physical characteristic is particularly useful in the gas chromatography of some lower boiling TMS-amino acids and TMS Krebs cycle acids.

**PROTOCOL**

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

**NOTE:** This protocol is not recommended for sugars.

**Ordering Information**

<table>
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<th>Description</th>
<th>Pkg. Size</th>
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<tbody>
<tr>
<td>TS-38828</td>
<td>BSTFA ((N,O-\text{bis(trimethylsilyl)}) trifluoroacetamide)</td>
<td>25 g Hypo-Vial Sample Storage Vial</td>
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<tr>
<td>TS-38829</td>
<td>BSTFA</td>
<td>100 g Hypo-Vial™ Sample Storage Vial</td>
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<tr>
<td>TS-38830</td>
<td>BSTFA</td>
<td>10 x 1 ml ampules</td>
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</table>

**BSTFA + TMCS**

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

\[
\begin{align*}
\text{CH}_3 & \quad \text{BSTFA} \\
\text{CH}_3 & \quad \text{MW 257.4} \\
\text{O} & \quad \text{bp 40°C/12 mm} \\
\text{CF}_3 & \quad \text{d}^\circ 0.961 \\
\end{align*}
\]

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**

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<td>TS-38831</td>
<td>BSTFA + 1% TMCS ((N,O-\text{bis(trimethylsilyl)}\text{trifluoroacetamide + 1% Trimethylchlorosilane}))</td>
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<tr>
<td>TS-38832</td>
<td>BSTFA + 1% TMCS</td>
<td>10 g Hypo-Vial Sample Storage Vial</td>
</tr>
<tr>
<td>TS-38833</td>
<td>BSTFA + 1% TMCS</td>
<td>25 g Hypo-Vial Sample Storage Vial</td>
</tr>
<tr>
<td>TS-38834</td>
<td>BSTFA + 1% TMCS</td>
<td>100 g Hypo-Vial Sample Storage Vial</td>
</tr>
<tr>
<td>TS-38840</td>
<td>BSTFA + 10% TMCS ((N,O-\text{bis(trimethylsilyl)}\text{trifluoroacetamide + 10% Trimethylchlorosilane}))</td>
<td>10 x 1 ml ampules</td>
</tr>
</tbody>
</table>
MSTFA and MSTFA 1% TMCS

Offers maximum volatility.

\[
\begin{align*}
\text{MSTFA} & \quad \text{MW: 199.1} \\
\text{bp: 70°C/75 mm} & \quad \text{d}^2_\text{4} \quad 1.11
\end{align*}
\]

**Highlights:**
- Trimethylsilyl donor strength comparable to BSA and BSTFA
- Reacts to replace labile hydrogens on a wide range of polar compounds with a -Si(CH\textsubscript{3})\textsubscript{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**

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.

**BSA**

The perfect reagent for volatile TMS derivatives.

\[
\begin{align*}
\text{BSA} & \quad \text{MW: 203.4} \\
\text{bp: 71-73°C/35 mm} & \quad \text{d}^2_\text{4} \quad 0.832
\end{align*}
\]

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 amines to form N-TMS derivatives

**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 unconjugated 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 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 70°C for 15 minutes.
4. Analyze by gas chromatography.

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

**Ordering Information**

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<td>TS-48910</td>
<td>MSTFA (N-Methyl-N-trimethylsilyl trifluoroacetamide)</td>
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<tr>
<td>TS-48911</td>
<td>MSTFA</td>
<td>10 g</td>
</tr>
<tr>
<td>TS-48913</td>
<td>MSTFA</td>
<td>25 ml</td>
</tr>
<tr>
<td>TS-48914</td>
<td>MSTFA</td>
<td>100 ml</td>
</tr>
<tr>
<td>TS-48915</td>
<td>MSTFA + 1% TMCS (N-Methyl-N-trimethylsilyl trifluoroacetamide + 1% Trimethylchlorosilane)</td>
<td>10 x 1 ml ampules</td>
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</table>

- Additional hazardous handling charge.
MTBSTFA and MTBSTFA + 1% TBDMCS

Offers stable TBDMS (tert-butyldimethylsilyl) derivatization.

<table>
<thead>
<tr>
<th>Product #</th>
<th>Description</th>
<th>Pkg. Size</th>
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<tr>
<td>TS-48920</td>
<td>MTBSTFA (N-Methyl-N-[(tert-butyldimethylsilyl)-trifluoroacetamide)</td>
<td>5 ml</td>
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<tr>
<td>TS-48927</td>
<td>MTBSTFA +1% TBDMCS</td>
<td>10 x 1 ml</td>
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Additional hazardous handling charge.

Highlights:
- Derivatizes hydroxyl, carboxylic acid, and amine groups
- 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
- Silylation potential increased by adding 1% TBDMCS

Thermo Scientific Pierce N-Methyl-N-[(tert-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) provides TBDMS derivatives without the disadvantage of earlier reported TBDMS-CI 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.)

TMSI

The strongest hydroxyl silylating agent 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 carbonyl groups, 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 bears 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. Cap vial and heat at 100°C for 2 hours.
3. Add 0.2 ml Pierce TMSI.
4. Cap vial and heat at 60°C for 3 hours.
5. Add 0.1 ml HFBI, TEAI or PFPI (depending on which acyl derivative is desired).
6. Cap vial and heat at 60°C for 30 minutes.
7. 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 sample, 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. Cap vial and heat at 100°C for 2 hours.
4. Analyze by gas chromatography.

Protocol 3

This method combines TMSI and pyridine for wet sugars. (DMF is not recommended for primary or secondary amines.)

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

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<td>TS-88623</td>
<td>TMSI (N-Trimethylsilylimidazole)</td>
<td>10 x 1 ml ampules</td>
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<td>TS-88625</td>
<td>TMSI</td>
<td>25 g Hypo-Vial Sample Storage Vial</td>
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<tr>
<td>TS-88626</td>
<td>TMSI</td>
<td>100 g Hypo-Vial Sample Storage Vial</td>
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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 hydrolysates.

**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**

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<td>TS-84770</td>
<td>HMDS (Hexamethyldisilazane)</td>
<td>25 g Hypo-Vial Sample</td>
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<td>TS-84769</td>
<td>HMDS</td>
<td>100 g Hypo-Vial Sample</td>
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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.

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<tr>
<td>TS-86530</td>
<td>TMCS</td>
<td>25 g bottle</td>
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*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 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.

**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.

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**Tri-Sil HTP (HDMS: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:

$$3 \text{ ROH} + \text{Me}_3\text{SiNHSiMe}_3 \rightarrow \text{Me}_3\text{SiCl} + 3 \text{ROSiMe}_3 + \text{NH}_4\text{Cl}$$

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.

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<td>✖ TS-48999</td>
<td>Tri-Sil HTP Reagent HMDS:TMCS:Pyridine (2:1:10)</td>
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<tr>
<td>TS-49001</td>
<td>Tri-Sil HTP Reagent HMDS:TMCS:Pyridine (2:1:10)</td>
<td>50 ml Hypo-Vial Sample Storage Vial</td>
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✖ 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**

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<td>TS-49012</td>
<td>Tri-Sil BP Reagent (2.5 mEq/ml BSA in pyridine)</td>
<td>25 ml Hypo-Vial Sample Storage Vial</td>
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* Additional hazardous handling charge.

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**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 cortisol. 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.

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<td>Tri-Sil TBT Reagent TMSi:BSA:TMCS (3:3:2)</td>
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* Additional hazardous handling charge.
Tri-Sil TP Reagent (TMSI:pyridine)

Great for derivatizing hydroxyl compounds.

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

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

PROTOCOL 1

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

PROTOCOL 2

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

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

Ordering Information

<table>
<thead>
<tr>
<th>Product #</th>
<th>Description</th>
<th>Pkg. Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS-49230</td>
<td>Tri-Sil TP Reagent</td>
<td>10 x 1 ml ampules</td>
</tr>
<tr>
<td>TS-49231</td>
<td>Tri-Sil TP Reagent</td>
<td>25 ml Hypo-Vial Sample Storage Vial</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Product #</th>
<th>Description</th>
<th>Pkg. Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS-20862</td>
<td>Acetonitrile</td>
<td>50 ml Hypo-Vial Sample Storage Vial</td>
</tr>
<tr>
<td>TS-20672</td>
<td>Dimethylformamide</td>
<td>50 ml Hypo-Vial Sample Storage Vial</td>
</tr>
<tr>
<td>TS-20684</td>
<td>Dimethylsulfoxide</td>
<td>50 ml Hypo-Vial Sample Storage Vial</td>
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<tr>
<td>TS-27530</td>
<td>Pyridine</td>
<td>50 ml Hypo-Vial Sample Storage Vial</td>
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<tr>
<td>TS-27860</td>
<td>Tetrahydrofuran</td>
<td>50 ml Hypo-Vial Sample Storage Vial</td>
</tr>
</tbody>
</table>

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

For more information, or to download product instructions, visit thermo.com/columns