Introduction

Accurate identification of proteins and analysis of post-translation modifications by mass spectrometry require accurate and complete protein digestion and peptide modification. The Thermo Scientific™ In-Solution Tryptic Digestion and Guanidination Kit contains optimized procedures and reagents for reduction/alkylation, digestion and guanidination to provide reliable mass spectrometry results.

Note: Guanidination requires trifluoroacetic acid (TFA, Product No. 28904), which must be purchased separately.

Procedure Summary

1. Denature/reduce at 95°C for 5 minutes
2. Alkylate in the dark at room temperature for 20 minutes
3. Digest at 37°C for 5 hours
4. Guanidinate at 65°C for 12 minutes

Important Product Information

- Trypsin is a serine protease that specifically cleaves bonds at the carboxyl side of lysine and arginine residues; however, cleavage can be slowed or blocked in a sequence-dependent manner (i.e., near proline residue, or with multiple adjoining cut sites). Peptide fragments with one missed cut are common and should be considered during mass analysis.

- The Thermo Scientific™ Pierce™ Trypsin Protease, MS Grade displays limited autolytic activity that should not interfere with mass spectral analysis. Using standard conditions, the most common trypsin fragment has a mass of 842.51 (m/z, M + H). The Modified Trypsin can be used as an internal standard.

- Reduction and alkylation of cystine residues using DTT and iodoacetamide (IAA), respectively, will minimize the appearance of unknown masses from disulfide bond formation and side-chain modification and improve detection of cystine-containing peptides. Alkylation with IAA increases the mass of a peptide by 57.02 for each cystine present.
Because arginine side chains are basic, peptides with terminal arginine residues ionize more efficiently and are detected more readily. To enhance overall ionization, guanidination is necessary to convert lysines to homoarginines. This derivatization results in improved signal from lysine-containing tryptic peptide ions. Guanidination increases peptide mass by 42.0 for each modified lysine.

**Additional Materials Required**

- Trifluoroacetic acid (TFA, Product No. 28904)
- 0.5mL microcentrifuge tubes
- Ultrapure water [18 megaohm (MΩ) equivalent]

**Note:** Use ultrapure water in the preparation of all materials.

**Material Preparation**

**Note:** Some of the solutions required for the procedure require occasional preparation while others require preparation just before use, therefore, plan accordingly.

<table>
<thead>
<tr>
<th>Material</th>
<th>Preparation Details</th>
</tr>
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<tbody>
<tr>
<td>Trypsin Stock</td>
<td>Add 20µL of the supplied Trypsin Storage Solution to the vial containing the lyophilized Pierce Trypsin Protease, MS Grade. To minimize freeze-thaw cycles and for optimal stability, divide the Trypsin Stock solution into four separate tubes, each containing ~5µL. Store aliquots at -20°C in a nonfrost-free freezer. <strong>Note:</strong> The Trypsin Storage Solution contains components that inactivate and protect the enzyme from autodigestion.</td>
</tr>
<tr>
<td>Activated Trypsin</td>
<td>Thaw a Trypsin Stock aliquot on ice. Dilute stock 10-fold by adding 45µL of ultrapure water for a final concentration of ~100ng/µL. Store this solution at -20°C for up to two months.</td>
</tr>
<tr>
<td>Digestion Buffer</td>
<td>Weigh 10mg of the Ammonium Bicarbonate and dissolve it in 2.5mL of ultrapure water for a final concentration of ~50mM. Store this solution at 4°C for up to two months.</td>
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<tr>
<td>Reducing Buffer</td>
<td>Puncture foil covering of the Thermo Scientific™ No-Weigh™ DTT tube with an empty pipette tip. Resuspend contents of tube with 500µL of ultrapure water for a final concentration of ~100mM. Transfer solution to a labeled microcentrifuge tube. This solution provides sufficient Reducing Buffer for ~330 reactions. Store Reducing Buffer at -20°C.</td>
</tr>
<tr>
<td>Alkylation Buffer</td>
<td>Prepare Alkylation Buffer just before use. Weigh 9mg of Iodoacetamide (IAA) and add it to a foil-wrapped tube to avoid exposure to light. Add 500µL of ultrapure water to the IAA for a final concentration of ~100mM. This is sufficient Alkylation Buffer for 166 reactions. Excess IAA is supplied in this kit. <strong>Note:</strong> Do not store Alkylation Buffer.</td>
</tr>
<tr>
<td>Guanidination Reagent</td>
<td>Weigh 50mg O-Methlyisourea hemisulfate and dissolve it in 51µL of ultrapure water. The final volume will be ~80µL and is enough Guanidination Reagent for ~13 reactions. Store excess reagent at -20°C.</td>
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</tbody>
</table>

**Procedure for In-solution Digestion and Guanidination**

**A. Reduction and Alkylation**

1. Add 15µL of Digestion Buffer and 1.5µL of Reducing Buffer to a 0.5mL microcentrifuge tube.
2. Add ≤ 10.5µL of a solution containing 0.025-10µg of protein to the tube and adjust the final volume to 27µL with ultrapure water.
3. Incubate sample at 95°C for 5 minutes. Allow sample to cool.
4. Prepare Alkylation Buffer as described in the Material Preparation Section. Add 3µL of Alkylation Buffer to the tube and incubate in the dark at room temperature for 20 minutes.
B. Digestion
1. Prepare Activated Trypsin as described in the Material Preparation Section.
2. Add 1µL of Activated Trypsin to the reaction tube and incubate at 37°C for 3 hours.
3. Add an additional 1µL of Activated Trypsin to the reaction tube and incubate at 37°C for 2 hours. Alternatively, incubate reaction overnight at 30°C.

C. Guanidination
1. The digested sample may be divided at this time to provide a no guanidination control, or the entire digest may be guanidinated. To divide digest, place 16µL of the digest to a new tube and label as no guanidination control. To the remaining digest add 16µL of ultrapure water.
2. Add 10µL of Ammonium Hydroxide to the digest. Vortex to mix well.
3. Add 6µL of Guanidination Reagent and vortex to mix well. Incubate sample at 65°C for 12 minutes.
4. Add 3µL of TFA to stop reaction, or proceed directly to sample preparation appropriate for downstream analysis.

Note: If required, the stopped reaction may be stored at -20°C for an extended period before analysis.
5. Guanidinated sample is now ready for liquid chromatographic separation or electrospray ionization mass spectrometry (LC-MS). If matrix-assisted laser desorption ionization (MALDI) mass spectrometry is to be performed, C18 clean-up may be required (e.g., Thermo Scientific™ Pierce™ C18 Spin Columns, Product No. 89870).

Troubleshooting

<table>
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<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incomplete digestion</td>
<td>Insufficient enzyme activity</td>
<td>Increase incubation time</td>
</tr>
<tr>
<td></td>
<td>Enzyme activity decreased during storage</td>
<td>Use a new Trypsin Stock aliquot</td>
</tr>
<tr>
<td>Poor mass spectrum</td>
<td>Concentration or detection limits of application</td>
<td>Ensure sample is within detection limit of the specific downstream application</td>
</tr>
<tr>
<td></td>
<td>Interfering agents</td>
<td>Clean-up digest with a C18 sample prep device</td>
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</table>

Related Thermo Scientific Products

- 89871 In-Gel Tryptic Digestion Kit
- 89870 Pierce C18 Spin Columns, 25/pkg
- 28904 Trifluoroacetic Acid, Sequanal Grade, 10 x 1mL
- 88300 Pierce Fe-NTA Phosphopeptide Enrichment Kit

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