INSTRUCTIONS

Thermo SCIENTIFIC

In-Solution Tryptic Digestion and Guanidination Kit

89895

1730.1

Number	Description
89895	**In-Solution Tryptic Digestion and Guanidination Kit,** sufficient reagents for ~90 digests of samples containing 0.025-10µg of protein

Kit Contents:

Pierce™ Trypsin Protease, MS Grade, 20µg

Trypsin Storage Solution, 50µL

Ammonium Bicarbonate, 50mg

No-Weigh™ DTT, 7.7mg

Iodoacetamide (IAA), 500mg

***O*-Methylisourea Hemisulfate Salt (OMI),** 400mg

Ammonium Hydroxide (30%), 1mL

Storage: Upon receipt store the Pierce Trypsin Protease at -20°C in a nonfrost-free freezer; store other components at 4°C. Kit is shipped on gel pack.

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.

Trypsin Stock	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. **Note:** The Trypsin Storage Solution contains components that inactivate and protect the enzyme from autodigestion.
Activated Trypsin	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.
Digestion Buffer	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.
Reducing Buffer	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.
Alkylation Buffer	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. **Note: Do not store Alkylation Buffer.**
Guanidination Reagent	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.

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

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

Related Thermo Scientific Products

89871 **In-Gel Tryptic Digestion Kit**

89870 **Pierce C18 Spin Columns,** 25/pkg

28904 **Trifluoroacetic Acid, Sequanal Grade,** 10 × 1mL

88300 **Pierce Fe-NTA Phosphopeptide Enrichment Kit**

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