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Prepare SILAC peptides using the In-Gel Tryptic Digestion Kit

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Introduction

The Pierce SILAC Protein Quantitation Kits with RPMI 1640 (Product No. 89982) or DMEM (Product No. 89983) contain all reagents for successful isotope metabolic protein labeling, enabling quantitation of protein expression levels from differentially treated cell populations. Stable isotope labeling with amino acids in cell culture (SILAC) is a simple and accurate method to quantify differential changes in the proteome.[1-4] SILAC uses metabolic incorporation of non-radioactive ^{13}C- or ^{15}N-labeled amino acids, referred to as 'heavy' amino acids, into proteins using specially formulated media.

Typical SILAC experiments involve growing two cell populations that are identical except that one contains the natural (light isotope) and the other contains the heavy isotope amino acid (e.g., $^{12}C_6$ and $^{13}C_6$ L-lysine, respectively). Protein levels in one sample are then altered through chemical treatment or genetic manipulation, and equal amounts of cell lysate from both cell populations are combined for sample processing and subsequent protein separation by SDS-PAGE. Proteins are digested with trypsin to generate peptides for MS analysis and quantitation of isotopic peptide pairs.

Several Thermo Scientific protein/peptide sample enrichment and preparation products are useful components in this overall experimental system. One of these is the Pierce In-Gel Tryptic Digestion Kit (Product No. 89871). This Tech Tip describes a modified version of the default In-Gel Tryptic Digestion Kit procedure, optimized for applications such as SILAC that require processing several gel pieces comprising an entire 1-D gel lane instead of selected small spots from a 2-D gel.

Protocol for Using the In-Gel Tryptic Digestion Kit on a Large Scale

Note: This protocol uses the entire 20 µg of Modified Trypsin provided in the In-Gel Tryptic Digestion Kit (Product No. 89871) to prepare peptides from approximately 20 gel pieces, each 7.5 × 7.5 mm (represents pieces comprising approximately two complete lanes of a typical 1-D polyacrylamide protein gel).

A. Additional Materials Required

- Clean polypropylene tubes, pipette tips, etc.
- Clean razor blades
- 37°C incubator with shaker
- Formic acid (Product No. 28905)

B. Material Preparation:

Note: Make all solutions and buffers immediately before use in clean polypropylene tubes; do not store.

- Destaining Solution: Dissolve 80 mg of ammonium bicarbonate in 40 ml 50% acetonitrile in ultrapure water.
- Digestion Buffer: Dissolve 30 mg of ammonium bicarbonate in 15 ml ultrapure water.
- Reducing Buffer: Dilute 200 µl of TCEP in 2 ml Digestion buffer.
- Alkylation Buffer: Dissolve 60 mg of iodoacetamide (IAA) in 3 ml Digestion buffer.
- Activated Trypsin Solution: Add 20 µl of Trypsin Storage Solution to the 20 µg of lyophilized Modified Trypsin. Dilute solution with 180 µl of ultrapure water. Add 1.8 ml Digestion Buffer to diluted trypsin (makes 2 ml total volume).
- 5% formic acid: Dilute 500 µl of 100% formic acid with 5 ml of ultrapure water.

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C. Procedure

1. Cut excised gel squares (7.5 × 7.5 mm) into 2 × 2 mm pieces.
2. Add 400 µl Destaining Solution to the gel pieces. Incubate sample at 37°C for 30 minutes with shaking. Remove and discard Destaining Solution. Repeat this step twice or until gel slices are completely destained.
3. Add 100 µl of Reducing Buffer to the tube to completely cover gel slices and incubate at 60°C for 10 minutes.
4. Allow samples to cool; then remove and discard Reducing Buffer from tube.
5. Add 100 µl of Alkylation Buffer to the tube and incubate sample in the dark at room temperature for 1 hour.
6. Remove and discard Alkylation Buffer from tube.
7. Wash the sample by adding 400 µl Destaining Buffer to the tube. Incubate sample at 37°C for 15 minutes with shaking. Remove and discard Destaining Buffer from tube. Repeat this step once.
8. Shrink gel pieces by adding 200 µl of acetonitrile. Incubate gel pieces for 15 minutes at room temperature.
9. Carefully remove acetonitrile and allow gel pieces to air-dry for 15 minutes.
10. Swell gel pieces by adding 100 µl of Activated Trypsin solution to the tube. Incubate sample at room temperature for 15 minutes.
11. Add 250 µl Digestion Buffer to the tube. Incubate sample at 37°C for 4 hours or at 30°C overnight with shaking.
12. Remove digestion mixture and place in a new tube.
13. To further extract peptides, add 100 µl 5% formic acid solution to gel pieces and incubate for 5 minutes.
14. Remove formic acid solution and add to digestion mixture from Step 12.
15. Add 100 µl acetonitrile to gel pieces and incubate 37°C for 15 minutes with shaking.
16. Remove acetonitrile solution and add to it digestion mixture from Step 12. Lyophilize tryptic-digested peptides (i.e., the digestion mixture) to near dryness and reconstitute with 10 µl of 0.1% formic acid.
17. Proceed to MS analysis of peptides.

Related Pierce Products

89982	**Pierce SILAC Protein Quantitation Kit – RPMI 1640**
89983	**Pierce SILAC Protein Quantitation Kit – DMEM**
28905	**Formic Acid,** 10 × 1 ml
24582	**E-Zinc® Reversible Stain Kit,** sufficient for staining up to 20 SDS-PAGE mini-gels
24590	**GelCode™ Blue Stain Reagent,** 500 ml, sufficient for staining up to 25 SDS-PAGE mini-gels
24600	**SilverSNAP® Stain for Mass Spectrometry,** sufficient for silver staining 20 gels mini-gels and destaining 500 gel pieces for subsequent in-gel digestion and processing for mass spectrometry
89898	**In-Solution Tryptic Digestion and Guandination Kit,** sufficient for 90 digests
89853	**Phosphopeptide Isolation Kit,** 30 spin columns
89870	**PepClean™ C-18 Spin Columns,** 25/pkg

Current versions of product instructions are available at *www.thermo.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.