INSTRUCTIONS

PIERCE
3747 N. Meridian Road
P.O. Box 117
Rockford, IL 61105

Cell Surface Protein Isolation Kit

89881

1511.3

Number	Description
89881	**Cell Surface Protein Isolation Kit**, contains sufficient reagents for the biotinylation and isolation of mammalian cell surface proteins from eight samples consisting of four 90-95% confluent T75 flasks

Kit Contents:

EZ-Link® Sulfo-NHS-SS-Biotin, 8 × 12 mg vials

Quenching Solution, 16 ml

Lysis Buffer, 4.5 ml

Immobilized NeutrAvidin™ Gel, 2.25 ml settled gel supplied as 50% slurry (4.5 ml total volume)

Wash Buffer, 34 ml

Column Accessory Pack, 10 spin columns with bottom caps and 20 collection tubes

No-Weigh™ Dithiothreitol (DTT), 8 × 7.7 mg microtubes

BupH™ Phosphate Buffered Saline Pack, 2 packs, each pack results in 0.1 M sodium phosphate, 0.15 M NaCl; pH 7.2 when reconstituted with 500 ml of ultrapure water

BupH™ Tris Buffered Saline Pack, 1 pack, results in 0.025 M Tris, 0.15 M NaCl; pH 7.2 when reconstituted with 500 ml of ultrapure water

Storage: Upon receipt store the Sulfo-NHS-SS-Biotin at -20°C. Store all other components at 4°C. Product shipped at ambient temperature.

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Introduction

The Cell Surface Protein Isolation Kit enables convenient biotinylation and isolation of cell surface proteins for Western blot analysis. In this simple method, mammalian cells are first labeled with EZ-Link® Sulfo-NHS-SS-Biotin (Figure 1), a thiol-cleavable amine-reactive biotinylation reagent. Cells are subsequently lysed with a mild detergent and the labeled proteins are then isolated with Immobilized NeutrAvidin™ Gel (agarose beads). The bound proteins are released by incubating with SDS-PAGE sample buffer containing 50 mM DTT.

Cell surface proteins represent a key subset of the cell, most notably because of the high concentration of integral membrane proteins. These proteins play major roles in signal transduction, cell adhesion and ion transport and serve as common pharmacological targets.

This easy-to-use kit provides all the necessary components for optimal labeling and the subsequent isolation of this important group of proteins. Buffers are supplied pre-formulated to produce consistent results. The membrane-impermeable Sulfo-NHS-SS-Biotin reagent forms a stable covalent linkage with an extended spacer arm to reduce steric hindrances associated with avidin binding. The protocol is optimized for diverse mammalian cell lines, including HeLa, NIH3T3 and C6 and is useful for differential expression analysis between treated and non-treated cells or between one or more cell lines.

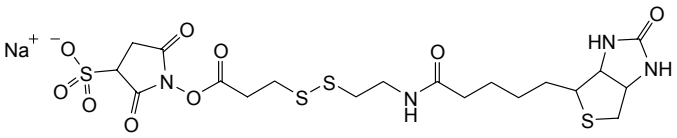
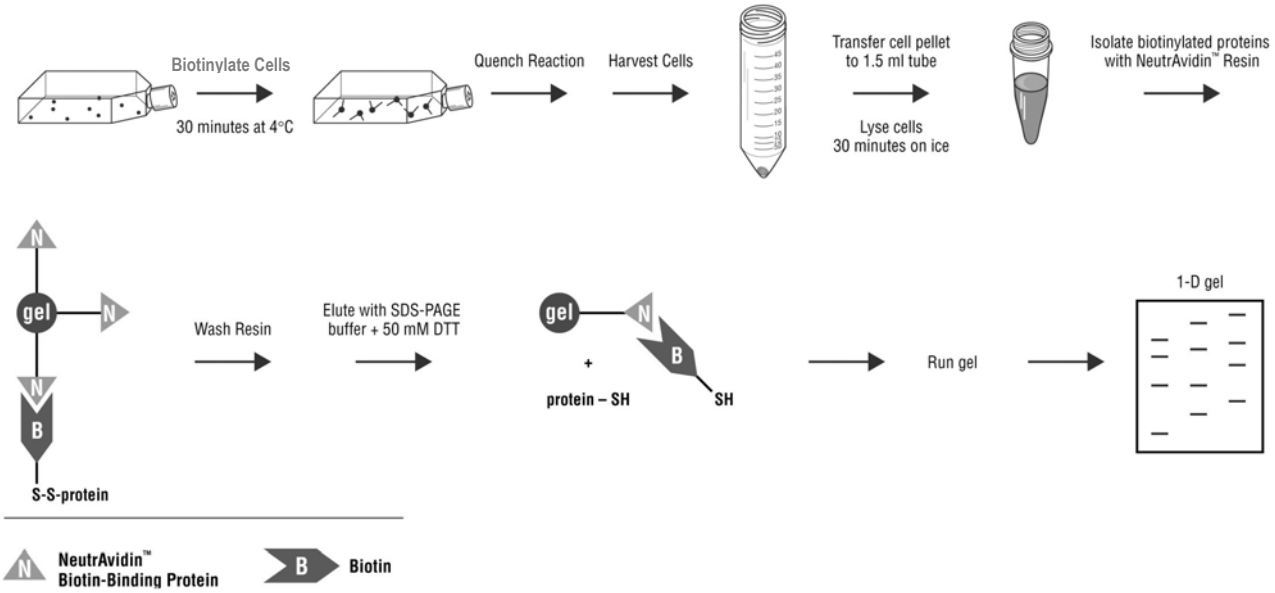


Figure 1. Chemical structure of Sulfo-NHS-SS-Biotin [sulfosuccinimidyl-2-(biotinamido)ethyl-1,3-dithiopropionate].

Procedure Summary



Additional Materials Required

- Sonicator (e.g., Misonix® Sonicator 3000 with microtip) (Optional)
- Sample rotator (e.g., Labquake® Shaker by Thermolyne) or rocking platform
- Microcentrifuge and 1.5 ml microcentrifuge tubes
- Centrifuge with swinging bucket or fixed angle rotor that can accommodate 50 ml conical tubes
- 50 ml conical tubes
- Protease inhibitors (e.g., Halt™ Protease Inhibitor Cocktail Kit, Product No. 78410)
- SDS-PAGE Sample Buffer (e.g., 62.5 mM Tris•HCl, pH 6.8, 1% SDS, 10% glycerol, Product No. 39001)
- Rocking platform or orbital shaker
- Cell scrapers

Material Preparation

Phosphate Buffered Saline (PBS)	Dissolve the dry-blend buffer with 500 ml of ultrapure water. For long-term storage of excess buffer, sterile filter the solution and store at 4°C.
Tris Buffered Saline (TBS)	Dissolve the dry-blend buffer with 500 ml of ultrapure water. For long-term storage of excess buffer, sterile filter the solution and store at 4°C.

Procedure for Cell Surface Biotinylation

Note: Not every protein on the cell surface will be extracted with this kit. Steric hindrance, lack of primary amines, and/or minimal sequence with extra-cellular exposure may prevent or interfere with labeling.

A. Biotinylation

1. Prepare four T75 cm^2 flasks of 90-95% confluent cells.

   Note: If cells are grown in suspension, use 1×10^7 cells per milliliter of the biotin solution prepared in step 3. Do not exceed a total of 4×10^7 cells per labeling reaction.

2. Remove media and wash cells twice with 8 ml of ice-cold PBS per flask. Quickly remove the PBS.

   Note: Do not allow PBS to remain in contact with cells for more than 5 seconds to prevent rounding and detachment of cells.

3. Dissolve the contents of one vial of Sulfo-NHS-SS-Biotin in 48 ml of ice-cold PBS. Add 10 ml of the biotin solution to each flask.
4. Place flasks on rocking platform or orbital shaker and gently agitate for 30 minutes at 4°C. This step ensures even coverage of the cells with the labeling solution.
5. Add 500 µl of Quenching Solution to each flask to quench the reaction. Gently tip the flask back and forth to ensure even coverage of the solution.
6. Gently scrape cells into solution and transfer the contents of all four flasks to a single 50 ml conical tube. Rinse all four flasks with a single 10 ml volume of TBS and add rinse volume to transferred cells.
7. Centrifuge cells at 500 × *g* for 3 minutes and discard supernatant.
8. Add 5 ml TBS to the cell pellet and gently pipette cells up and down twice with a serological pipette. Centrifuge at 500 × *g* for 3 minutes and discard supernatant.

B. Cell Lysis

1. Add protease inhibitors to 500 µl of Lysis Buffer and add it to the cells. Transfer cells in the lysis solution to a 1.5 ml microcentrifuge tube.
2. Pipette up and down to suspend the cells.
3. Using low power (e.g., 1.5) to prevent foaming, disrupt cells by sonicating on ice using five 1-second bursts.
4. Incubate cells 30 minutes on ice, vortexing every 5 minutes for 5 seconds. To improve solubilization efficiency, perform additional sonications during incubation.
5. Centrifuge cell lysate at 10,000 × *g* for 2 minutes at 4°C.
6. Transfer clarified supernatant to a new tube.

C. Isolation of Labeled Proteins

1. Insert a column into a collection tube.
2. Gently swirl the bottle of Immobilized NeutrAvidin™ Gel to obtain an even suspension. Add 500 µl of Immobilized NeutrAvidin™ Gel slurry to the column and cap the column.
3. Centrifuge 1 minute at 1,000 × *g* and discard flow-through. Reuse the collection tube through Step C.11.
4. Add 500 µl of Wash Buffer to the gel, centrifuge for 1 minute at 1,000 × *g* and discard flow-through. Repeat this step twice.

5. Apply bottom cap to column, add clarified cell lysate to the gel, and then apply top cap to column.

   Note: Make sure top and bottom caps are tightly in place.

6. Incubate for 60 minutes at room temperature with end-over-end mixing using a rotator. Alternatively, rock back and forth on a rocking platform.

7. Remove top cap and then bottom cap from column. Place column in the collection tube, and replace top.

   Note: Remove top cap before bottom cap to prevent lysate from leaking from the bottom of the column.

8. Centrifuge column for 1 minute at 1,000 × *g* and discard flow-through.

9. Add protease inhibitors to 2.5 ml of Wash Buffer.

10. Return column to the collection tube and add 500 µl Wash Buffer. Cap the column and mix by inverting the column. Centrifuge for 1 minute at 1,000 × *g*. Discard rinse and remove top cap. Repeat this step three times.

11. Replace bottom cap on column.

D. Protein Elution

1. Puncture the foil covering of one No-Weigh™ DTT Microtube with a pipette tip, and add 50 µl of ultrapure water to yield 1 M DTT.

2. Add 23.7 µl of the DTT solution to 450 µl SDS-PAGE Sample Buffer to make a final concentration of 50 mM DTT.

3. Add 400 µl of the Sample Buffer containing the DTT to the gel and cap the column. Incubate the reaction for 60 minutes at room temperature with end-over-end mixing on a rotator or rock back and forth on a rocking platform.

   Alternatively, place column in a new collection tube and heat in a heat block for 5 minutes at 95°C. Ensure that bottom cap is on tightly. Heating will cause recovery of some NeutrAvidin™ Protein monomer (15K) in the eluate. The monomer is not released when elution is performed at room temperature.

4. Remove the column's top cap first and then the bottom cap. Place column in a new collection tube and replace top cap.

5. Centrifuge column for 2 minutes at 1,000 × *g*.

6. Add a trace amount of bromophenol blue to eluate and analyze by Western blot. Store sample at -20°C if not used immediately.

Troubleshooting

Problem	Cause	Solution
Not recovering complex membrane proteins with multiple transmembrane domains	Proteins not well-solubilized during cell lysis	Sonicate more frequently during cell lysis (Step B.4) to improve recovery of proteins that are difficult to solubilize
Intracellular proteins are recovered in the eluate	Confluence of the cells was too great or cell integrity was compromised	Harvest cells when they are 90-95% confluent

Related Pierce Products

78410 **Halt™ Protease Inhibitor Cocktail Kit**

25200-25244 **Precise™ Protein Gels** (see catalog or web site for a complete listing)

34080 **SuperSignal® West Pico Chemiluminescent Substrate,** 500 ml

34075 **SuperSignal® West Dura Extended Duration Substrate,** 100 ml

34095 **SuperSignal® West Femto Maximum Sensitivity Substrate,** 100 ml

21059 **Restore™ Western Blot Stripping Buffer,** 500 ml

21065 **Erase-It® Background Eliminator Kit,** for eliminating background from X-ray film

34090 **CL-XPosure™ Film (5" × 7"),** 100 sheets

34091 **CL-XPosure™ Film (8"× 10"),** 100 sheets

References

DeBlaquiere, J. and Burgess, A.W. (1999). Affinity purification of the plasma membranes. *J. Biomol. Tech.* **10:**64-71.

Ellerbroek, S.M., *et al.* (2001). Functional interplay between type I collagen and cell surface matrix metalloproteinase activity. *J. Biol. Chem.* **276(27):**24833-42.

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Schuberth, H.-J., *et al.* (1996). Biotinylation of cell surface MHC molecules: a complementary tool for the study of MHC class II polymorphism in cattle. *J. Immunol. Methods*. **189:**89-98.

Wilchek, M. and Bayer, E.A. (1987). Labeling glycoconjugates with hydrazide reagents. *Methods in Enzymol.* **138:**429-42.

Current versions of all product instructions are available at *www.piercenet.com*. For a faxed copy call 800-874-3723) or contact your local distributor.