

Pierce Cell Surface Protein Isolation Kit

89881

1511.7

Number	Description
89881	<p>Pierce 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</p> <p>Kit Contents:</p> <p>EZ-Link Sulfo-NHS-SS-Biotin, 8 × 12mg vials</p> <p>Quenching Solution, 16mL</p> <p>Lysis Buffer, 4.5mL</p> <p>NeutrAvidin Agarose, 2.25mL settled gel supplied as 50% slurry (4.5mL total volume)</p> <p>Wash Buffer, 34mL</p> <p>Column Accessory Pack, 10 spin columns with bottom caps and 20 collection tubes</p> <p>Dithiothreitol (DTT), No-Weigh Format, 8 × 7.7mg microtubes</p> <p>BupH Phosphate Buffered Saline Pack, 2 packs, each pack results in 0.1M sodium phosphate, 0.15M NaCl; pH 7.2 when reconstituted with 500mL of ultrapure water</p> <p>BupH Tris Buffered Saline Pack, 1 pack, results in 0.025M Tris, 0.15M NaCl; pH 7.2 when reconstituted with 500mL of ultrapure water</p>

Storage: Upon receipt store kit at 4°C. Product shipped at ambient temperature.

Table of Contents

Introduction	2
Procedure Summary.....	2
Additional Materials Required.....	2
Material Preparation	3
Procedure for Cell Surface Biotinylation.....	3
A. Biotinylation	3
B. Cell Lysis	3
C. Isolation of Labeled Proteins	3
D. Protein Elution	4
Troubleshooting.....	4
Related Thermo Scientific Products	4
General References	5

Introduction

The Thermo Scientific™ Pierce™ 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 Thermo Scientific™ 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 Thermo Scientific™ NeutrAvidin™ Agarose. The bound proteins are released by incubating with SDS-PAGE sample buffer containing 50mM 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.

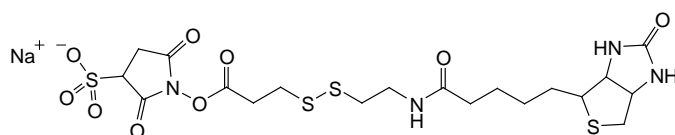
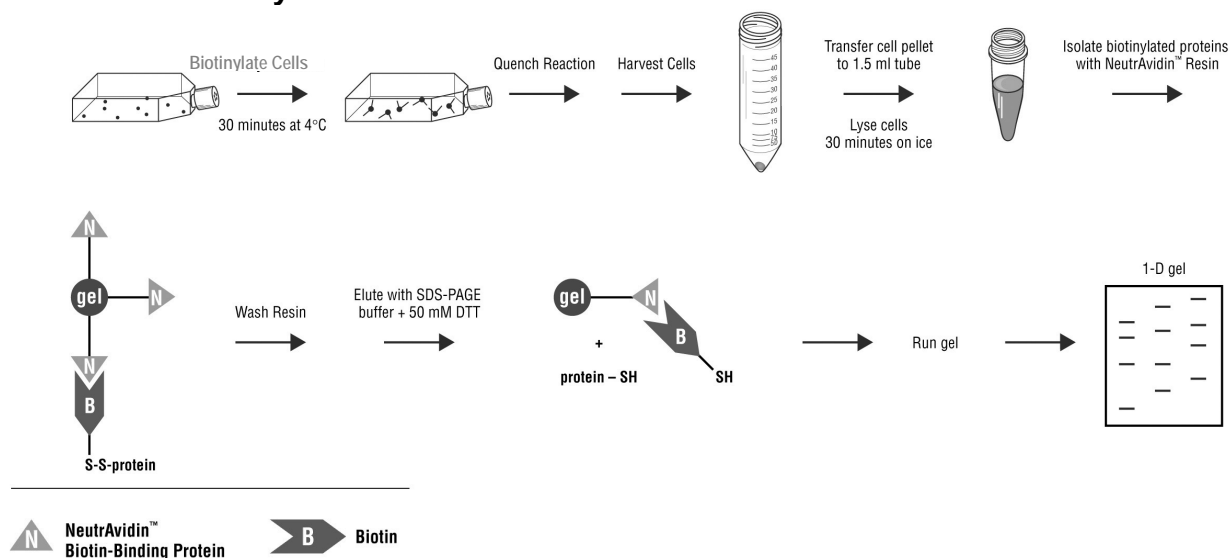


Figure 1. Chemical structure of Sulfo-NHS-SS-Biotin [sulfosuccinimidyl-2-(biotinamido)ethyl-1,3-dithiopropionate].

Procedure Summary



Additional Materials Required

- Sonicator (e.g., Sonicator™ Q700 with microtip by QSonica, LLC) (Optional)
- Sample rotator (e.g., Labquake™ Shaker) or rocking platform
- Microcentrifuge and 1.5mL microcentrifuge tubes
- Centrifuge with swinging bucket or fixed angle rotor that can accommodate 50mL conical tubes
- 50mL conical tubes
- Protease inhibitors (e.g., Halt™ Protease Inhibitor Cocktail Kit, Product No. 78410)
- SDS-PAGE Sample Buffer (e.g., 62.5mM 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 500mL 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 500mL 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 cm² flasks of 90-95% confluent cells.
Note: If cells are grown in suspension, use 1×10^6 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 8mL 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 48mL of ice-cold PBS. Add 10mL 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 500µL 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 50mL conical tube. Rinse all four flasks with a single 10mL volume of TBS and add rinse volume to transferred cells.
7. Centrifuge cells at $500 \times g$ for 3 minutes and discard supernatant.
8. Add 5mL TBS to the cell pellet and gently pipette cells up and down twice with a serological pipette. Centrifuge at $500 \times g$ for 3 minutes and discard supernatant.

B. Cell Lysis

1. Add protease inhibitors to 500µL of Lysis Buffer and add it to the cells. Transfer cells in the lysis solution to a 1.5mL 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 $10,000 \times g$ 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 NeutrAvidin Agarose to obtain an even suspension. Add 500µL of the NeutrAvidin Agarose slurry to the column and cap the column.
3. Centrifuge 1 minute at $1000 \times g$ and discard flow-through. Reuse the collection tube through Step C11.

4. Add 500µL of Wash Buffer to the gel, centrifuge for 1 minute at 1000 × 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 1000 × g and discard flow-through.
9. Add protease inhibitors to 2.5mL of Wash Buffer.
10. Return column to the collection tube and add 500µL Wash Buffer. Cap the column and mix by inverting the column. Centrifuge for 1 minute at 1000 × 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 50µL of ultrapure water to yield 1M DTT.
2. Add 23.7µL of the DTT solution to 450µL SDS-PAGE Sample Buffer to make a final concentration of 50mM DTT.
3. Add 400µL 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 1000 × 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 B4) 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 Thermo Scientific Products

- 20291** **Dithiothreitol (DTT), No-Weigh Format**, 48 microtubes
- 21331** **EZ-Link Sulfo-NHS-SS-Biotin**, 100mg
- 29200** **NeutrAvidin Agarose Resin**, 5mL

General 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.
- Jang, J.H. and Hanash, S. (2003). Profiling of the cell surface proteome. *Proteomics* **3**:1947-54.
- 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.

Products are warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation"). No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than Buyer. Any model or sample furnished to Buyer is merely illustrative of the general type and quality of goods and does not represent that any Product will conform to such model or sample.

NO OTHER WARRANTIES, EXPRESS OR IMPLIED, ARE GRANTED, INCLUDING WITHOUT LIMITATION, IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR ANY PARTICULAR PURPOSE, OR NON INFRINGEMENT. BUYER'S EXCLUSIVE REMEDY FOR NON-CONFORMING PRODUCTS DURING THE WARRANTY PERIOD IS LIMITED TO REPAIR, REPLACEMENT OF OR REFUND FOR THE NON-CONFORMING PRODUCT(S) AT SELLER'S SOLE OPTION. THERE IS NO OBLIGATION TO REPAIR, REPLACE OR REFUND FOR PRODUCTS AS THE RESULT OF (I) ACCIDENT, DISASTER OR EVENT OF FORCE MAJEURE, (II) MISUSE, FAULT OR NEGLIGENCE OF OR BY BUYER, (III) USE OF THE PRODUCTS IN A MANNER FOR WHICH THEY WERE NOT DESIGNED, OR (IV) IMPROPER STORAGE AND HANDLING OF THE PRODUCTS.

Unless otherwise expressly stated on the Product or in the documentation accompanying the Product, the Product is intended for research only and is not to be used for any other purpose, including without limitation, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses, or any type of consumption by or application to humans or animals.

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

© 2013 Thermo Fisher Scientific Inc. All rights reserved. Sonicator is a trademark of QSonica, LLC. Unless otherwise indicated, all other trademarks are property of Thermo Fisher Scientific Inc. and its subsidiaries. Printed in the USA.