Pierce™ Cell Surface Protein Biotinylation and Isolation Kit

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Thermo Scientific™ Pierce™ Cell Surface Protein Biotinylation and Isolation Kit enables biotinylation and isolation of cell surface proteins for Western blot analysis or mass spectrometry applications. Mammalian cells (adherent or in suspension) 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 detergent and the labeled proteins are then isolated with Thermo Scientific™ NeutrAvidin™ Agarose. The bound, labeled proteins are released by reduction of the disulfide bond with 10 mM DTT. The appendix includes information on how to prepare samples for mass spectrometry (MS) analysis.

Cell surface proteins represent a key subset of cellular proteins. They play major roles in signal transduction, cell adhesion, and ion transport and serve as common pharmacological targets. These proteins are challenging to efficiently extract and isolate due to their multiple spanning domains in the plasma membrane.

This easy-to-use kit provides all the necessary components for optimal labeling and 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 adherent and suspension cells, and is useful for differential expression analysis between treated and non-treated cells or between one or more cell lines.

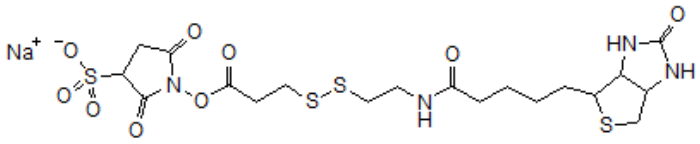


Fig. 1 Chemical structure of Sulfo-NHS-SS-Biotin [sulfosuccinimidyl-2-(biotinamido) ethyl-1,3-dithiopropionate].

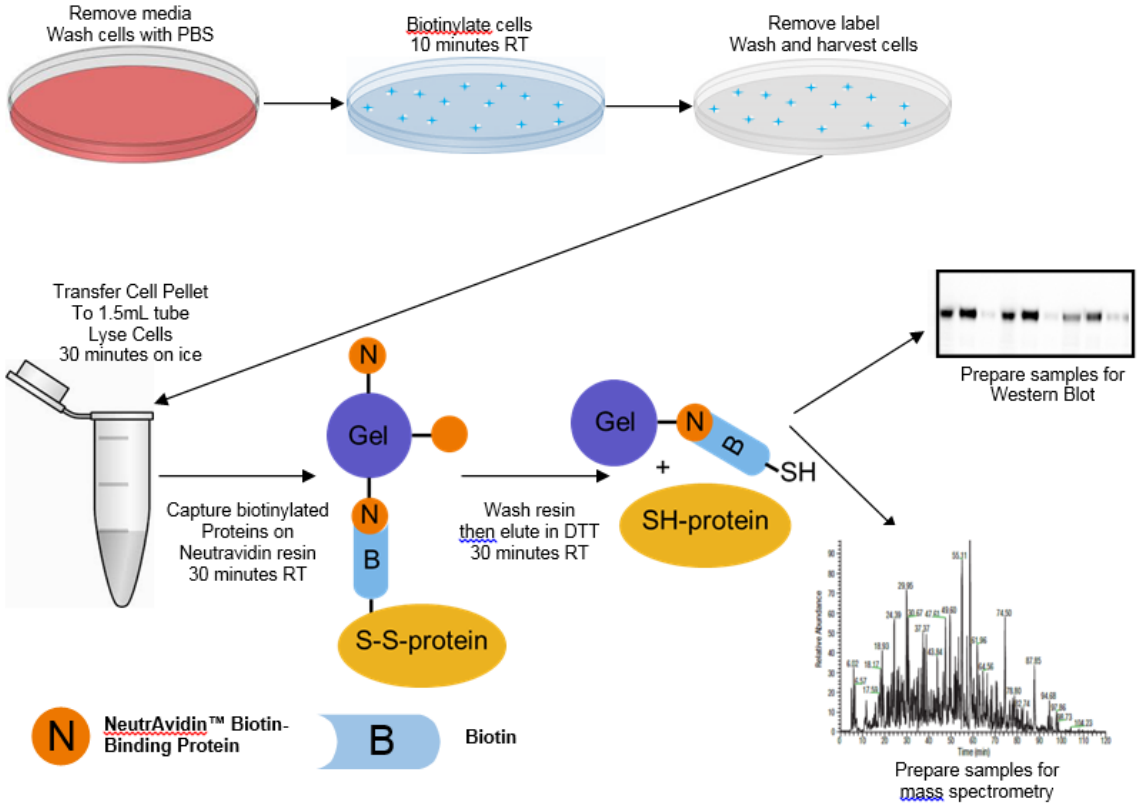
Contents

Table 1 Pierce™ Cell Surface Protein Biotinylation and Isolation Kit

Product No.	Kit Contents [1]	Amount	Storage
A44390	EZ-Link™ Sulfo-NHS-SS-Biotin	8 × 6 mg vials	Store Wash Buffer and Elution Buffer at room temperature and remaining kit at 4°C.
	Lysis Buffer	10 mL	
	NeutrAvidin™ Agarose	2.25 mL settled gel supplied as 50% slurry (4.5 mL total volume)	
	Wash Buffer	35 mL	
	Elution Buffer	5 mL	
	Column Accessory Pack	16 spin columns, 16 caps, 16 bottom plugs, 16 collection tubes	
	Dithithreitol (DTT), No-Weight Format	7.7 mg microtube	
	BupH™ Phosphate Buffered Saline Packs	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 Packs	2 packs, each pack results in 0.025 M Tris, 0.15 M NaCl; pH 7.2 when reconstituted with 500 mL of ultrapure water	

[1] Contains sufficient reagents for the biotinylation and isolation of mammalian cell surface proteins from 8 samples consisting of two 85-95% confluent 15 cm dishes or four T75 cm^2 flasks.

Procedure summary



Important product information

- Store Wash and Elution Buffers at room temperature.
- Addition of protease or protease/phosphatase inhibitors is recommended before cell lysis.
- Equal cell numbers across multiple plates can be difficult to obtain. To normalize the amount of sample across 2 or more plates, measure the protein concentration of clarified cell lysate from each plate (in "Lyse cells" on page 3), then add equal amounts of protein to the NeutrAvidin™ agarose (in "Isolate labeled proteins" on page 3). The Lysis Buffer is compatible with the Pierce™ BCA Protein Assay Kit (Product No. 23225).

Materials required but not provided

- Sample rotator (e.g., Labquake™ Shaker)
- Microcentrifuge and 1.5 mL microcentrifuge tubes (**Note:** If samples will be used for MS analysis, use low protein binding tubes, e.g., Thermo Scientific™ Low Protein Binding Collection Tubes, 1.5 mL, Product No. 90410)
- 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 (100X), Product No. 87786 or Halt™ Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100X), Product No. 78441)
- Cell scrapers
- For MS applications, TEAB (Product No. 90114) and the EasyPep™ Mini MS Sample Prep Kit (Product No. A40006)

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 room temperature.
- 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.
- DTT: Remove cap from tube of No-Weigh DTT and add 0.5 mL purified water. Pipet up and down until the DTT is fully dissolved. The stock concentration of DTT is 100 mM. Aliquot 30 µL DTT stock into each of 16 microcentrifuge tubes. Store DTT aliquots at -20°C for 1 year.

Procedure for cell surface biotinylation

Note:

- Not every protein on the cell surface will be extracted with this kit. Steric hindrance, lack of exposed primary amines, and/or minimal sequence with extra-cellular exposure may prevent or interfere with labeling.
- The protocol is written for one 15-cm dish of adherent cells or 9 × 10^6 to 2.4 × 10^7 suspension cells. Prepare 2 dishes per sample for a total of 8 samples or prepare 1 dish per sample for a total of 16 samples.
- One vial of Sulfo-NHS-SS-Biotin is dissolved into 24 mL of PBS, which is sufficient for labeling two 15 cm dishes (1 sample).
- Two T75 cm^2 flasks can be substituted for one 15 cm dish.
- For a negative control, omit the labeling reagent and incubate with PBS alone.

Biotinylate adherent cells

1. Prepare one 15 cm dish of >85% confluent cells. See table below for the approximate number of cells per dish of example cell lines.

Cell Line	Cell Number Per 15 cm Dish
HeLa	8 × 10^6 to 4 × 10^7
A549	6.5 × 10^6 to 4 × 10^7
HEK293	1 × 10^7 to 4 × 10^7

2. Remove media and wash cells with 20 mL of ambient PBS per plate. Quickly remove the PBS.
3. Dissolve the contents of one vial of Sulfo-NHS-SS-Biotin in 24 mL of ambient PBS. Add 10 mL of the biotin solution to one dish.

   Note: If processing an odd number of plates, suspend the contents of one vial of label in only 1 mL PBS to make a 24X

stock, transfer 0.5 mL into 11.5 mL of PBS and immediately freeze the remaining 0.5 mL at -20°C. It is NOT recommended to freeze larger volumes of dissolved label due to increased risk for hydrolysis when thawing.

4. Incubate dish for 10 minutes at room temperature. Ensure biotin solution completely covers the cells.
5. Remove labeling solution from dish and wash cells twice with 20 mL ice-cold TBS. Remove TBS after each wash.
6. Add 10 mL ice-cold TBS to dish and scrape cells into solution using the flat side of the scraper.

   Note: Ensure cells are scraped off the plate by looking at them under a microscope or viewing the underside of the plate while holding it under a light.
7. Transfer scraped cells into a 50 mL conical tube on ice. Immediately rinse scraped dish with a single 10 mL volume of TBS and add rinse volume to transferred cells.
8. Centrifuge cells at 500 × *g* for 3 minutes at 4°C and discard supernatant. Continue to "Lyse cells" on page 3.

Biotinylate suspension cells

Note: In order to maintain cell integrity and to minimize background contamination, take care when resuspending cell pellets.

1. Grow cells in suspension to 6×10^5 to 1.6×10^6 cells/mL.
2. Transfer 15 mL of cells to a 15 mL conical tube.
3. Centrifuge cells at 300 × *g* for 3 minutes and discard media.
4. Quickly and **GENTLY** resuspend cell pellet with 15 mL of ambient PBS.
5. Centrifuge cells at 300 × *g* for 3 minutes. Discard wash.
6. Dissolve the contents of one vial of Sulfo-NHS-SS-Biotin in 24 mL of ambient PBS. Add 10 mL of the biotin solution to tube and **GENTLY** resuspend the cells.

   Note: If processing only 1 tube of cells, suspend the contents of 1 vial of label in only 1 mL PBS to make a 24X stock, transfer 0.5 mL into 11.5 mL of PBS and immediately freeze the remaining 0.5 mL at -20°C. It is NOT recommended to freeze larger volumes of dissolved label due to increased risk for hydrolysis when thawing.
7. Incubate tube for 10 minutes at room temperature.
8. Centrifuge the cells at 300 × *g* for 3 minutes and remove label. Resuspend cells in 15 mL ice-cold TBS.
9. Centrifuge cells at 300 × *g* for 3 minutes and discard wash. Repeat TBS wash once. Continue to "Lyse cells" on page 3.

Lyse cells

1. Add protease inhibitors to 500 µL of Lysis Buffer and add it to the cell pellet.
2. Pipette up and down 20 times to suspend the cells.
3. Transfer cells in the lysis solution to a 1.5 mL microcentrifuge tube.
4. Incubate cells 30 minutes on ice, vortexing for 5 seconds at the beginning and end of the incubation.
5. Centrifuge cell lysate at 15,000 × *g* for 5 minutes at 4°C.
6. Transfer clarified supernatant to a new 1.5 mL tube.

Isolate labeled proteins

1. Twist off bottom tip from one column.
2. Insert column into a collection tube.
3. Gently swirl the bottle of NeutrAvidin™ Agarose to obtain an even suspension. Using a scissors, cut off the end of a p1000 pipet tip to widen the borehole. Add 250 µL of the NeutrAvidin™ Agarose slurry to the column and screw on top cap, loosened one-quarter turn before centrifuging.
4. Centrifuge 1 minute at 1,000 × *g* and discard storage solution. Reuse the collection tube through step 9 below.
5. Apply bottom plug to column, add clarified cell lysate to the resin, and then apply top cap to column.

   Note: Make sure top and bottom caps are tightly in place.
6. Incubate for 30 minutes at room temperature with end-over-end mixing on a rotator. Alternatively, rock back and forth on a rocking platform.
7. Loosen top cap and then remove bottom cap from column. Place column in the collection tube.

   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 flowthrough.
9. Return column to the collection tube and add 500 µL Wash Buffer. Cap the column and mix by inverting the column (in collection tube) 2 to 3 times, while pushing on the cap and bottom of collection tube simultaneously. Centrifuge for 1 minute at 1,000 × *g*. Discard wash and remove top cap. Repeat this step 3 times for a total of 4 washes.
10. Replace bottom cap on column.

    Note: Biotinylated proteins on washed resin can be analyzed by MS using the sample preparation protocol provided in "Appendix A: Procedure for sample preparation for mass spectrometry (MS)" on page 4 below. This protocol for MS sample preparation is an alternative to the elution protocol for Western blotting provided in "Elute proteins" on page 3.

Elute proteins

1. Mix 225 µL Elution Buffer and 25 µL DTT stock solution. The final concentration of DTT is 10 mM. Add 200 µL of prepared Elution Buffer to the resin and cap the column. Incubate the reaction for 30 minutes at room temperature with end-over-end mixing on a rotator or rock back and forth on a rocking platform.
2. Loosen the top cap, remove the bottom cap and place into a collection tube.
3. Centrifuge column for 2 minutes at 1,000 × *g*.
4. Add sample buffer to the eluate and analyze by Western blot.

Troubleshooting

Observation	Possible cause	Recommended action
Yield of cell surface proteins is low.	Low number of cells or cells not scraped efficiently off the plate.	Harvest cells when they are 85-95% confluent or use 2 dishes of cells instead of 1. Ensure that cells have been removed from plate. If not, scrape again and rinse plate with TBS.
Cells lose adherency from the plate during incubation with label in PBS.	Some cell lines (e.g., HEK293) do not adhere well to the plate in PBS at room temperature.	Perform labeling step at 4°C for 30 minutes.
Poor recovery of complex membrane proteins with multiple transmembrane domains.	Proteins not well-solubilized during cell lysis.	Increase incubation time with Lysis Buffer or use more Lysis Buffer.

Observation	Possible cause	Recommended action
Intracellular proteins are recovered in the eluate.	Cell integrity was compromised or agarose gel was not washed sufficiently.	Ensure label was completely removed from the cells before scraping. Alternatively, perform additional washes on the agarose containing labeled proteins.

Appendix A: Procedure for sample preparation for mass spectrometry (MS)

Proteomic MS can be performed on isolated cell surface proteins as an alternative to Western blotting. Sample preparation for MS analysis is performed using the following protocol. Additional reagents are required.

Materials required from the cell surface kit

DTT (see "Material preparation" on page 2 for preparation of aliquots)

Other materials required but not provided

- EasyPep™ Mini MS Sample Prep Kit (Product No. A40006)
- 1M Triethylammonium bicarbonate (TEAB) for TMT experiments (Product No. 90114)
- LC/MS grade water or ultrapure water

Note: The following components in the EasyPep™ Mini MS Sample Prep Kit will NOT be used in this protocol:

- Universal nuclease
- Reduction Solution

Elute, reduce protein, and alkylate

This protocol was modified from the manual of the EasyPep™ Mini MS Sample Prep Kit making it compatible with the Cell Surface Biotinylation and Isolation Kit.

Prepare 100 mM triethylammonium bicarbonate (TEAB) by diluting 1 M TEAB 10 times using LC/MS grade water or ultrapure water (30 mL is sufficient for 16 samples).

1. (Continued from step 10 in "Isolate labeled proteins" on page 3) Return column to the collection tube and add 500 µL of 100 mM TEAB. 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 twice for a total of 3 washes.
2. Replace bottom cap on column.
3. Add 120 µL of purified water to a 30 µL aliquot of 100 mM DTT. The final concentration of DTT is 20 mM.
4. Add 100 µL of the Lysis Solution to the column. (Lysis Solution is in the EasyPep™ Mini MS Sample Prep Kit).
5. Add 100 µL of 20 mM DTT to the column. The final concentration of DTT will be 10 mM.
6. Incubate the reaction for 45 minutes at room temperature with end-over-end mixing on a rotator or rock back and forth on a rocking platform.
7. Loosen the column's top cap first and then remove the bottom cap. Place column in a new Low Protein Binding Collection Tube. (The Low Protein Binding Collection Tubes can be found in the EasyPep™ Mini MS Sample Prep Kit).
8. Centrifuge column for 2 minutes at 1000 × *g*. Discard the column and keep the flowthrough.
9. Add 50 µL of Alkylation Solution from the EasyPep™ Mini MS Sample Prep Kit.
10. Incubate sample at 37°C using a heat block for 30 minutes to alkylate the protein sample.

Digest protein

1. Add 500 µL of Enzyme Reconstitution Solution to 1 vial of Trypsin/Lys-C Protease Mix. (Both can be found in the EasyPep™ Mini MS Sample Prep Kit.)
2. Add 10 µL of the reconstituted enzyme solution to the reduced and alkylated protein sample solution.

   Note: Store unused reconstituted enzyme at 4°C for 1 month or -20°C for 1 year.
3. Incubate with shaking at 37°C for 1-3 hours to digest the protein sample.
4. After incubation is completed, add 40 µL of Digestion Stop Solution found in the EasyPep™ Mini MS Sample Prep Kit.

Clean-up peptides (same as in EasyPep™ Mini MS Sample Prep Kit)

1. Remove the white cap at the bottom of the peptide clean-up column, loosen the green top cap, and place into a 2 mL microcentrifuge tube. (Peptide clean-up column is in the EasyPep™ Mini MS Sample Prep Kit.)
2. Centrifuge at 3,000 × *g* for 2 minutes to remove all liquid from the column. Discard the flowthrough.
3. Transfer the protein digest sample (~300 µL total volume) into the dry peptide clean-up column.
4. Centrifuge at 1,500 × *g* for 2 minutes. Discard the flowthrough.
5. Add 300 µL of Wash Solution A into the column. (Wash Solution A is in the EasyPep™ Mini MS Sample Prep Kit.)
6. Centrifuge at 1,500 × *g* for 2 minutes. Discard the flowthrough.
7. Add 300 µL of Wash Solution B into the column. (Wash Solution B is in the EasyPep™ Mini MS Sample Prep Kit.)
8. Centrifuge at 1,500 × *g* for 2 minutes. Discard the flowthrough.
9. Transfer the peptide clean-up column into a new 2 mL microcentrifuge tube.
10. Add 300 µL of the Elution Solution into the column. (Elution Solution is in the EasyPep™ Mini MS Sample Prep Kit.)
11. Centrifuge at 1,500 × *g* for 2 minutes to collect the clean peptide sample.
12. Dry the peptide sample using a vacuum centrifuge.
13. Resuspend the sample in 40 µL of 0.1% formic acid in water for LC-MS analysis.
14. *(Optional)* Assess peptide yield and concentration using a quantitative peptide assay (e.g., Pierce™ Quantitative Colorimetric Peptide Assay Kit (Product No. 23275). Adjust the peptide concentration with 0.1% formic acid in water solution for optimal LC-MS column loading.

    Note: *(Optional)* Label peptides with TMT™ reagent - This protocol is compatible with TMT labeling. Refer to the manual for the EasyPep™ Mini MS Sample Prep Kit.

    Note: The cleaved biotin modification is CAMthiopropanoyl (MW = 145.020 Daltons).

    Note: For further troubleshooting and other information refer to the manual for the EasyPep™ Mini MS Sample Prep Kit.

Related products

Product	Product No.
Dithiothreitol (DTT), No-Weight Format, 48 × 7.7 mg	A39255
EZ-Link™Sulfo-NHS-SS-Biotin, 100mg	21331
NeutrAvidin™ Agarose Resin	29200

General references

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