Cell Surface Protein Isolation Kit

Cat. No. CSF-001K **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Cell surface proteins play a major role in signal transduction, cell adhesion, transport and serve as diagnostic and pharmacological targets. Our Cell Surface Protein Isolation Kit provides a simple and efficient method for the isolation of cell surface proteins. In this method, cells are first labeled with Sulfo-NHS-SS-Biotin, an amine-reactive, thiol-cleavable, biotinylation reagent. Cells are subsequently lysed and the labeled cell surface proteins are isolated using Streptavidin beads. The bound proteins are then released from beads by incubating with DTT solution. Biotinylation reagent is cell-membrane-impermeable with an extended spacer arm to reduce steric hindrances associated with streptavidin binding. This convenient kit provides all the required components for optimal labeling and isolation of the cell surface proteins.
Applications	Isolated cell surface proteins can be used for various downstream applications such as Western Blotting or other structural and functional studies
Storage	Store kit at 4centigrade. Briefly spin small vials prior to opening.
Kit Components	• Quenching Solution 10 ml • Lysis Buffer 6.5ml • Wash Buffer 12ml • Streptavidin Beads 1.5ml • PBS Tablet 3 • TBS Tablet 1 • Sulfo-NHS-SS-Biotin 5 vials • DTT 100 ul

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Materials Required but Not Supplied	Cell scrapers Orbital shaker and sample rotator Protease inhibitors SDS-PAGE sample buffer
Technical Notes	Sample Type: Adherent and suspension cells
Preparation	PBS Tablet: Dissolve one PBS Tablet in 100 ml of ddH2O to make 1X PBS solution. Sterile filter the solution and store at 4centigrade. TBS Tablet: Dissolve in 100 ml of ddH2O to make 1X TBS solution. Sterile filter the solution and store at 4centigrade. Sulfo-NHS-SS-Biotin: Each vial is good for two reactions. Reconstitute each vial with 2 ml of 1XPBS. Pipette up and down to completely dissolve the powder. Make working solution of Sulfo-NHS-SS-Biotin by adding 2 ml reconstituted solution to 18 ml of ice cold 1XPBS. Notes: a. Always reconstitute Sulfo-NHS-SS-Biotin vial just before use. b. Optional: Sulfo-NHS-SS-Biotin can also be reconstituted in anhydrous DMSO or DMF. Reconstitute the powder in 100 µl of DMSO or DMF and add to 19.9 ml of ice cold 1XPBS. DTT: Aliquot and store at -20centigrade. Use within 2 months.
Separation Protocol	1. Sample Preparation: Grow appropriate cells in desired media to >90% confluency in a T75 flask. Keep the flask on ice for 15 min. Remove media and wash cells with 10 ml of ice-cold 1X PBS. Notes: - We recommend quick washing of adherent cells as prolonged exposure to PBS will cause detachment of cells. - In case of suspension cells, centrifuge cells at 500 X g, 4centigrade to remove the media.

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2. Biotinylation:

a. After washing, add 10 ml of freshly prepared working solution of Sulfo-NHS-SS-Biotin (ice-cold) to the cells. Incubate with gentle agitation at 4centigrade for 30 min. After incubation, add 1 ml of Quenching Solution and incubate with gentle agitation at 4centigrade for 5 min.

b. Gently scrape the cells (for adherent cells), and collect in a 50 ml conical tube. Centrifuge cells at 500 X g, 4centigrade or room temperature (RT) for 3 min.

c. Remove the supernatant and wash cells twice by resuspending in 5 ml of 1X TBS. At this step, cells can be transferred to a 1.5 ml Eppendorf tube.

Note: Some cell surface proteins may not be biotinylated/isolated with this kit since steric hindrance, lack of primary amines, and/or minimal sequence with extra-cellular exposure may prevent or interfere with labeling.

3. Cell Lysis:

a. Resuspend cells in 400-500 µof Lysis Buffer with protease inhibitors (not provided) and incubate on ice for 30 min. Vortex briefly every 15 min. Spin the lysate at 10,000 X g for 2 min. at RT and collect the supernatant.

b. During cell lysis, take ~150 µof the provided 50% slurry of Streptavidin Beads for each reaction. Spin at 800 X g for 1 min. Remove the aqueous phase carefully. Equilibrate the Beads by resuspending in ~ 150 µof Lysis Buffer. Remove Lysis Buffer prior to use.

4. Binding of Labeled Proteins: Add the collected supernatant from step 3 to the packed equilibrated Streptavidin Beads. Incubate at RT for 1 hr. with end-over-end mixing on a rotator. Spin down the beads at 800 X g for 1 min. Save ~300 µof the supernatant as the unbound lysate. Take care not to disturb the beads.

5. Protein Elution:

a. Wash beads by adding 400 µWash Buffer. Spin at 800 X g for 1 min. and carefully remove the wash buffer without losing the beads. Wash beads 2 more times.

b. Prepare 100 µelution buffer for each reaction by adding 10 µof 1 M DTT to 90 µof 1X PBS. Add 100 µof elution buffer to the packed beads and incubate at RT for 30 min. with brief vortexing every 10 min.

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	c. Spin down the beads at room temperature and collect the supernatant as eluate containing the isolated cell surface proteins. Unbound lysate and isolated cell surface proteins can be stored at -20centigrade or -80centigrade for subsequent analysis.

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