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Cell Surface Protein Isolation Kit

(Catalog # K295-10; 10 reactions; Store at 4°C)

I. Introduction:

Cell surface proteins play a major role in signal transduction, cell adhesion, transport and serve as diagnostic and pharmacological targets. BioVision's 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.

II. Application:

- Isolated cell surface proteins can be used for various downstream applications such as Western Blotting or other structural and functional studies.

III. Sample Type:

- Adherent and suspension cells

IV. Kit Contents:

Components	K295-10	Cap Code	Part Number
Quenching Solution	10 ml	NM	K295-10-1
Lysis Buffer	6.5 ml	Blue	K295-10-2
Wash Buffer	12 ml	WM	K295-10-3
Streptavidin Beads	1.5 ml	Violet	K295-10-4
PBS Tablet	3	Clear	K295-10-5
TBS Tablet	1	WM	K295-10-6
Sulfo-NHS-SS-Biotin	5 Vials	Amber	K295-10-7
DTT	100 µl	Green	K295-10-8

V. User Supplied Reagents and Equipment:

- Cell scrapers
- Orbital shaker and sample rotator
- Protease inhibitors (Cat. # K272 or equivalent)
- SDS-PAGE sample buffer (Cat. # 2108 or equivalent)

VI. Storage Conditions and Reagent Preparation:

Store kit at 4°C. Briefly spin small vials prior to opening. Read entire protocol before performing the assay.

- **PBS Tablet**: Dissolve one PBS Tablet in 100 ml of ddH_2O to make 1X PBS solution. Sterile filter the solution and store at 4°C.
- **TBS Tablet**: Dissolve in 100 ml of ddH_2O to make 1X TBS solution. Sterile filter the solution and store at 4°C.
- **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 -20°C. Use within 2 months.

VII. Cell Surface Protein Isolation 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, 4°C to remove the media.

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 4°C for 30 min. After incubation, add 1 ml of Quenching Solution and incubate with gentle agitation at 4°C 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, 4°C 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 µl of 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 µl of 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 µl of 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 µl of the supernatant as the unbound lysate. Take care not to disturb the beads.
5. **Protein Elution**:
   a. Wash beads by adding 400 µl Wash 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 µl elution buffer for each reaction by adding 10 µl of 1 M DTT to 90 µl of 1X PBS. Add 100 µl of elution buffer to the packed beads and incubate at RT for 30 min. with brief vortexing every 10 min.
   c. Spin down the beads at room temperature and collect the supernatant as eluate containing the isolated cell surface proteins. **Note:** Unbound lysate and isolated cell surface proteins can be stored at -20°C or -80°C for subsequent analysis.

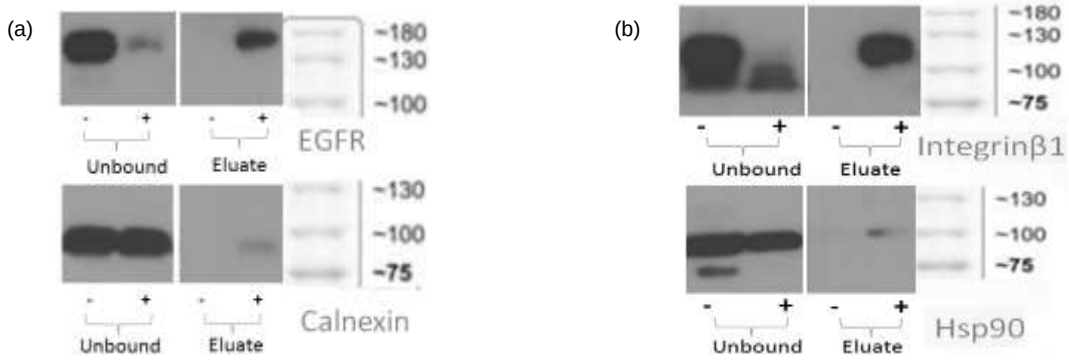

Figure: Western Blot analysis of isolated cell surface proteins: HeLa cells were used to isolate the cell surface proteins, in the presence (+) and absence (-) of Sulfo-NHS-SS-Biotin. (a) EGFR (Endoplasmic Growth Factor Receptor) as Plasma Membrane marker and Calnexin as Cytosolic marker protein and (b) Integrin β1 and Hsp90 as Plasma Membrane and Cytosolic markers, respectively. Assay was performed following the kit protocol.

VIII. Related Products:

Membrane Protein Extraction Kit (K268)
Mitochondria Isolation Kit for Tissue & Cultured Cells (K288)
Cytosol/Particulate Separation Kit (K267)
FractionPREP™ Cell Fractionation Kit (K270)
Immunoprecipitation (IP) Kit (K286)
Yeast Nuclei Isolation Kit (K289)
Mitochondrial Protein IP Kit (K285)
Mitochondria/Cytosol Fractionation Kit (K256)
Nuclear/Cytosol Fractionation Kit (K266)
Mammalian Cell Extraction Kit (K269)
Yeast Mitochondria Isolation Kit (K259)

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