ab206998
Cell surface protein isolation kit

Instructions for use:

For isolation of cell surface proteins, which can be used for various downstream applications such as western blotting or other structural and functional studies.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 6, Last Updated 26 April 2024

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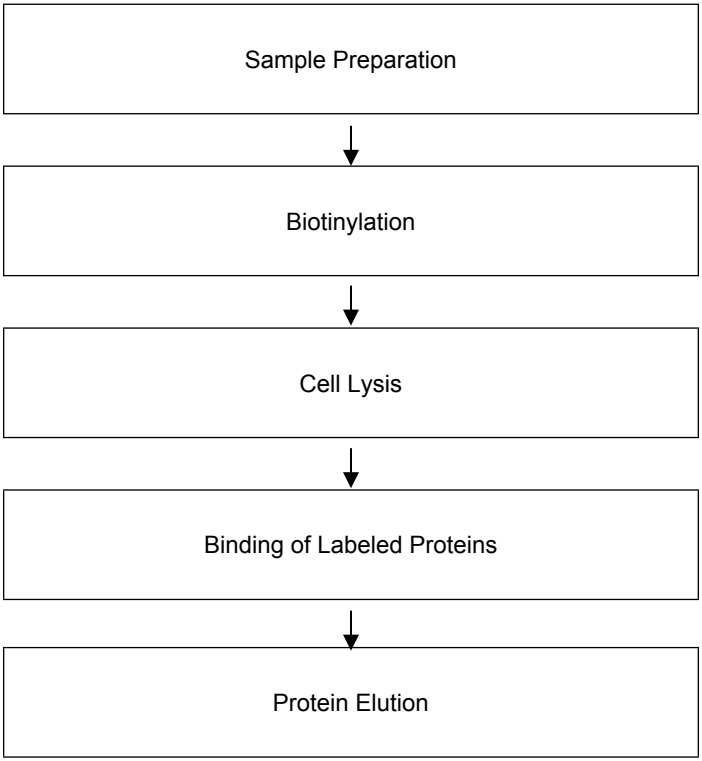
1. BACKGROUND

Abcam's Cell surface protein isolation kit (ab206998) enables isolation of cell surface proteins for various downstream applications such as western blotting and other structural and functional studies.

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

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 4°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 6 and 9.

5. LIMITATIONS

- Kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Quenching Solution I /Quenching Solution	10 mL	4°C	4°C
Lysis Buffer VIII/Lysis Buffer	6.5 mL	4°C	4°C
Wash Buffer VII/Wash Buffer	12 mL	4°C	4°C
Streptavidin Beads	1.5 mL	4°C	4°C
PBS Tablet	1	4°C	4°C
TBS Tablet	1	4°C	4°C
Sulfo-NHS-SS-Biotin	5 vials	4°C	4°C
DTT II/DTT	100 µL	-20°C	-20C

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- Cell scrapers
- Orbital shaker and sample rotator
- Protease inhibitors
- SDS-PAGE sample buffer

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single isolation experiment. The starting amount of tissue or cells for a single experiment will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Always reconstitute the Sulfo-NHS-SS-Biotin vial just before use.
- 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 1X PBS.
- We recommend quick washing of adherent cells as prolonged exposure to PBS will cause detachment of cells
- If using suspension cells, centrifuge cells at 500 x g, 4°C to remove the media.
- Some cell surface proteins may not be biotinylated/isolated by this kit since steric hindrance, lack of primary amines, and/or very short extracellular regions may prevent or interfere with labeling.

9. REAGENT PREPARATION

9.1. **Quenching Solution I /Quenching Solution:**

Ready to use as supplied.

9.2. **Lysis Buffer VIII/Lysis Buffer:**

Ready to use as supplied.

9.3. **Wash Buffer VII/Wash Buffer:**

Ready to use as supplied.

9.4. **Streptavidin Beads:**

Ready to use as supplied.

9.5. **PBS Tablet:**

Dissolve one PBS Tablet in 500 mL of ddH_2O to make 1X PBS solution. Sterile filter the solution and store at 4°C

9.6. **TBS Tablet:**

Dissolve in 100 mL of ddH_2O to make 1X TBS solution. Sterile filter the solution and store at 4°C.

9.7. **Sulfo-NHS-SS-Biotin:**

Each vial is good for two reactions. Reconstitute each vial with 2 mL of 1X PBS. 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 1X PBS. **Always reconstitute vial just before use.**

9.8. **DTT II/DTT:**

Aliquot and store at -20°C. Use within 2 months.

10. SAMPLE PREPARATION

10.1. Grow cells expressing the cell surface protein of interest in media of choice to >90% confluency in a T75 flask.

10.2. Keep the flask on ice for 15 minutes.

10.3. Remove media and wash cells with 10 mL of ice-cold 1X PBS.

11. ASSAY PROCEDURE

11.1. Biotinylation

11.1.1. After washing cells, 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 minutes. After incubation, add 1 mL of Quenching Solution I /Quenching Solution and incubate with gentle agitation at 4°C for 5 minutes.

11.1.2. Gently scrape the cells (for adherent cells), and collect in a 50 mL conical tube. Centrifuge cells at 500 x g, 4°C for 3 minutes.

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

11.2. Cell Lysis

11.2.1. Re-suspend cells in 400-500 µL of Lysis Buffer VIII/Lysis Buffer with protease inhibitors (not provided) and incubate on ice for 30 minutes. Vortex briefly every 15 minutes. Spin the lysate at 10,000 x g for 2 minutes at room temperature and collect the supernatant.

11.2.2. Meanwhile, prepare the Streptavidin Beads by spinning ~150 µL of the 50% slurry for each reaction at 800 x g for 1 minute. Remove the aqueous phase carefully. Equilibrate the beads by resuspending in ~ 150 µL of Lysis Buffer VIII/Lysis Buffer. Remove Lysis Buffer VIII/Lysis Buffer just before use.

11.3. Binding of Labeled Proteins

11.3.1. Add the supernatant collected in step 11.2.1 to the equilibrated Streptavidin Beads. Incubate at room temperature for 1 hour with end-over-end mixing on a rotator.

11.3.2. Spin down the beads at 800 x g for 1 minute. Remove as much supernatant (unbound lysate) as possible without disturbing the beads and transfer to a microcentrifuge tube. Keep the supernatant on ice for analysis at the end of the isolation experiment.

11.4. Protein Elution

11.4.1. Wash beads by adding 400 µL Wash Buffer VII/Wash Buffer. Spin at 800 x g for 1 minute and carefully remove the Wash Buffer VII/wash buffer without losing the beads. Wash beads 2 more times.

11.4.2. Prepare 100 µL elution buffer for each reaction by adding 10 µL of DTT II/1 M DTT to 90 µL of 1X PBS. Add 100 µL of elution buffer to the beads and incubate at room temperature for 30 minutes with brief gentle vortexing every 10 minutes.

11.4.3. Spin down the beads at room temperature and collect the supernatant (eluate), taking care not to disturb the beads. The eluate contains the isolated cell surface proteins.

11.4.4. Analyze the unbound lysate and isolated cell surface proteins by western blot to confirm that the protein of interest has been isolated.

Note: *Unbound lysate and isolated cell surface proteins can be stored at -20°C or -80°C for subsequent analysis.*

12. TYPICAL DATA

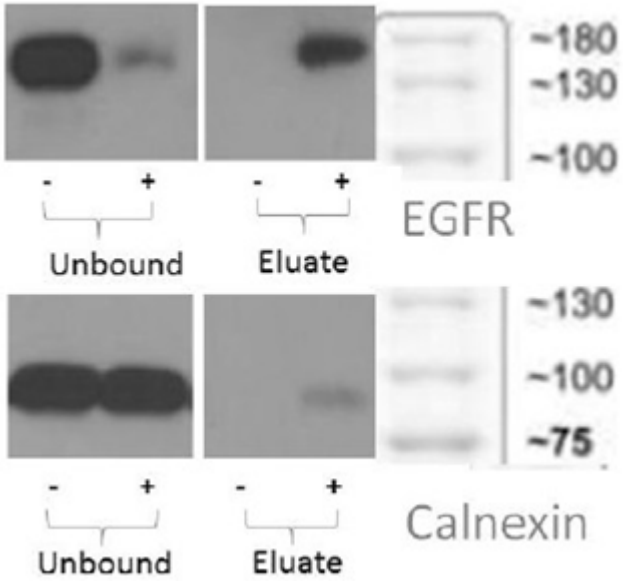


Figure 1: Western blot analysis of cell surface proteins isolated using ab206998 Cell surface proteins were isolated from HeLa cells in the presence (+) and absence (-) of Sulfo-NHS-SS-Biotin. The blots were probed with antibodies to Endoplasmic Growth Factor Receptor (EGFR), a plasma membrane marker (top) and Calnexin, a cytosolic marker (bottom).

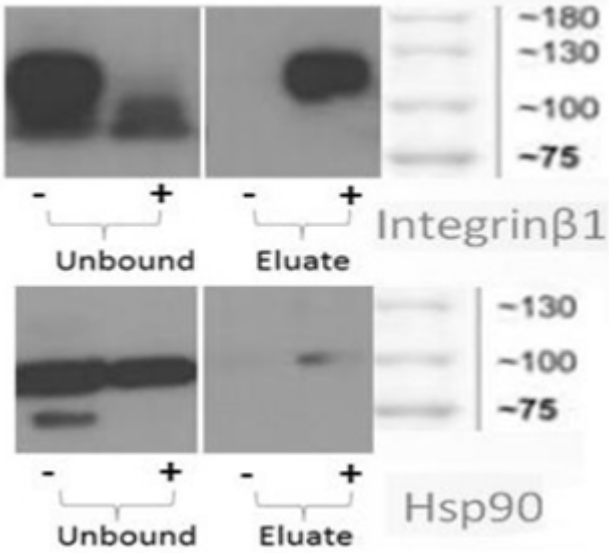


Figure 2: western blot analysis of isolated cell surface proteins HeLa cells were used to isolate the cell surface proteins, in the presence (+) and absence (-) of Sulfo-NHS-SS-Biotin. (b) Integrin β1 and Hsp90 as plasma membrane and cytosolic markers, respectively. Isolation was performed following the kit protocol.

13. NOTES

RESOURCES

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)