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A Geno Technology, Inc. (USA) brand name

HOOK™ Cell Surface Protein Isolation

(Cat. # 786-316)

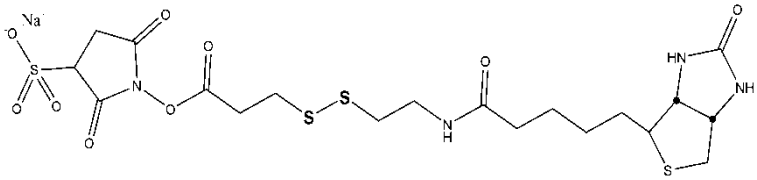


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INTRODUCTION

HOOK™ Cell Surface Protein Isolation kit uses G-Biosciences HOOK™ biotin labeling and purification technology in conjunction with our Mammalian Cell PE LB™ lysis buffer to conveniently label cell surface proteins and isolate them for further analysis, including Western blotting.

Mammalian cells, adherent or non-adherent, are first labeled with HOOK™ Sulfo-NHS-SS-Biotin. HOOK™ Sulfo-NHS-SS-Biotin is a water-soluble, amine reactive biotinylation reagent that has a *N*-hydroxysulfosuccinimide (sulfo-NHS) ester. The addition of a charged sulfonate (SO^{3-}) on the *N*-hydroxysuccinimide ring of the sulfo-NHS esters results in its solubility in water, but prevents it becoming permeable to plasma membranes. The solubility and impermeability to plasma membranes makes HOOK™ Sulfo-NHS-SS-Biotin ideal for studying cell surface proteins as it will only react with the protein molecules on the outer surface of plasma membranes. An additional advantage of HOOK™ Sulfo-NHS-SS-Biotin is the presence of a disulfide bond in the spacer arm. The disulfide bond permits the cleavage of the biotin moiety from the protein, making its interaction with avidin/ streptavidin reversible.



HOOK™ Sulfo-NHS-SS-Biotin

Following labeling cells are lysed with Mammalian Cell PE LB™, a buffered lysis buffer that employs a mild non-ionic detergent for enhanced extraction and stability of proteins, and the cell lysate is applied to a Streptavidin agarose column. Unlabeled intracellular proteins are washed away and the biotin labeled cell surface proteins are then released by reduction of the disulfide bond with DTT.

The kit is supplied with all the necessary reagents and buffers for convenience and improved reproducibility. The kit is compatible with a wide variety of mammalian cells and can be used to compare treated and untreated cells and differences between different cell lines. This kit is supplied with sufficient reagents for five experiments, with each experiment consisting of four 90-95% confluent T-75cm^2 flasks.

ITEM(S) SUPPLIED (Cat. # 786-316)

Description	Size
HOOK™-Sulfo-NHS-SS-Biotin	50mg
Streptavidin-Agarose column with stoppers	5
Collection Tube (2ml)	10
Mammalian Cell PE LB™	30ml
HOOK™ Quench Buffer	2 x 5ml
JAW™ Phosphate Buffered Saline Packs (1 liter)	1
OneQuant™ DTT	5 vials

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store it at 4°C.

ADDITIONAL ITEMS NEEDED

- Orbital shaker
- Protease inhibitor cocktail (e.g. G-Biosciences ProteaseArrest™, Cat. # 786-108)
- Sonicator

PROTOCOL

See Appendix for general scheme.

***NOTE**: HOOK™ Cell Surface Protein Isolation Kit isolates cell surface proteins with high efficiency, however due to steric hindrance, lack of primary amines, and/or short extra-cellular sequence exposure some proteins will not be isolated.*

I. Cell Surface Protein Biotinylation

1. a. Cultivate adherent cells in four T-75cm^2 flasks and grow to 90-95% confluency.
   b. For cell suspensions, use 1x10^7 cells per milliliter of biotin solution (prepared in step 3).
2. Wash the cells twice in 10ml ice-cold PBS per flask.
3. Dissolve 10mg HOOK™ Sulfo-NHS-SS-Biotin in 40 ml ice-cold PBS and add 10 ml of the biotin solution to each flask. See Step 1b for non-adherent cells.
   Warning: NHS Ester has limited stability in aqueous buffers. Ensure that PBS is ice cold before adding NHS Ester. Mix immediately prior to use and quickly add to cells.
4. Gently agitate the cells for 30 minutes at 4°C on an orbital shaker.
5. Add 0.5ml HOOK™ Quench Buffer to each flask and gently mix by rocking the flask back and forth a few times.
6. Gently scrape cells into solution and transfer the contents of the flasks to a 50ml conical tube. Rinse the flasks with a combined total volume of 10ml PBS and add it to the 50ml conical tube.
7. Pellet the cells at 500×*g* for 2 minutes and discard the supernatant.
8. Gently resuspend the cell pellet in 5ml PBS, centrifuge as before and discard supernatant.

II. Cell Lysis

1. Add appropriate protease inhibitors (e.g. G-Biosciences ProteaseArrest™) to 500µl Mammalian Cell PE LB™ and add to the cell pellet. Pipette up and down to resuspend and transfer the suspension to a 1.5ml centrifuge tube.
2. Sonicate the cells on ice with five 1 second pulses.
   NOTE*: Use a low sonication setting to prevent frothing.*
3. Following sonication, incubate cells on ice for 30 minutes for complete lysis. Vortex every 5 minutes for improved lysis.
4. Centrifuge lysates for 5 minutes at 10,000×*g* at 4ºC and transfer the supernatant to a fresh tube.

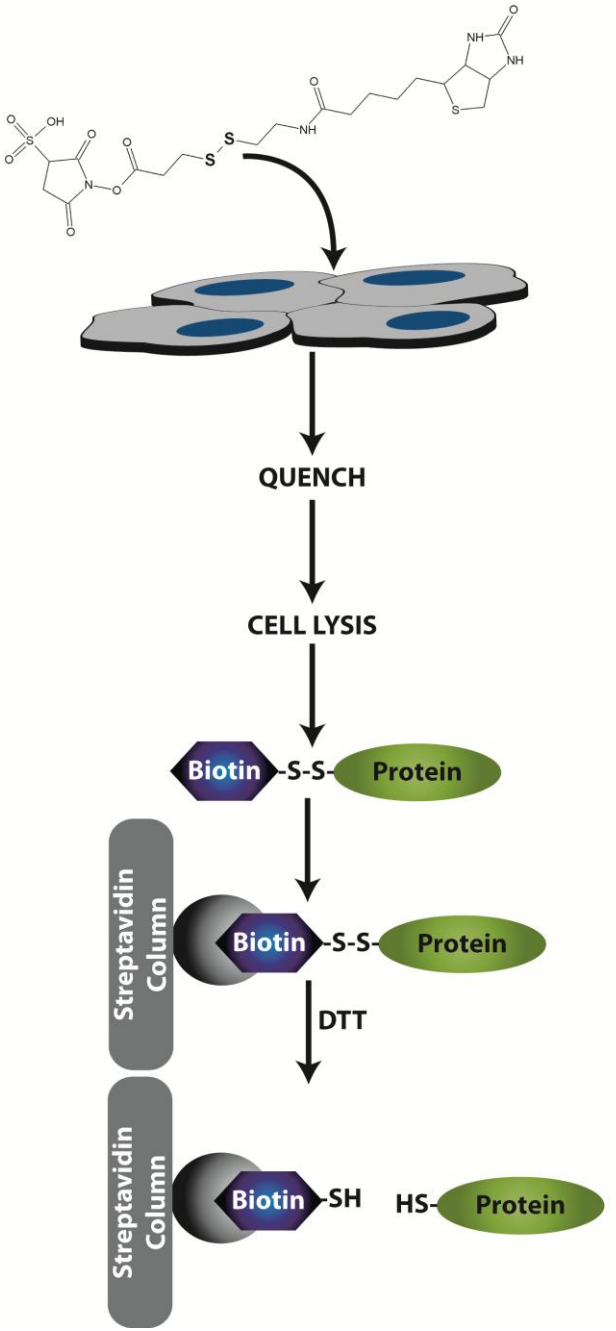
III. Isolation of Labeled Proteins

1. Remove the cap of a Streptavidin-Agarose Column and then snap off the bottom. Place the column in a 2ml collection tube. Centrifuge 1,000xg for 10 seconds and discard the flow through.
2. Wash the column: Add 500µl Mammalian Cell PE LB™ to the column, centrifuging at 1,000xg 10 seconds and discard the flow through. Repeat the washing one more time. Save the collection tube.
3. Apply the stopper to the bottom of the column and add 500µl lysate from Section II, step 4. Apply the cap to the column and incubate for 30-60 minutes at room temperature preferably on a rotator. Alternatively, rock back and forth on a rocking platform.
4. Remove the cap and then the stopper from the column and return the column to the 2ml collection vial. Centrifuge at 1000xg for 10 seconds and discard the flow through.
5. Wash the column 6 times with 500µl Mammalian Cell PE LB™ each time as described in step 2 above.

IV. Protein Elution

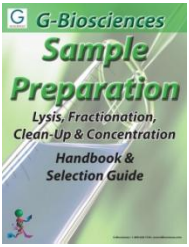
1. Add 90µl of Mammalian Cell PELB to one vial of OneQuant™ DTT and vortex until completely dissolved. Add 50µl DTT solution to 450µl Mammalian Cell PE LB™ in a tube and vortex to mix.
2. Apply the stopper to the bottom tip of the column and add 400µl Mammalian Cell PE LB™ containing DTT to the column and put the top cap.
3. Incubate the column for 30-60 minutes at room temperature preferably on a rotator. Alternatively, rock back and forth on a rocking platform.
4. Remove the top cap and then stopper from the column and place the column in a clean 2ml collection tube. Centrifuge at 1000xg for 10 seconds. The eluted cell surface proteins are now ready for further analysis e.g. Western blotting etc.

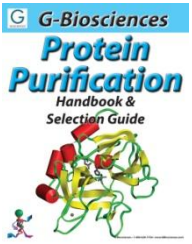
APPENDIX: GENERAL SCHEME



RELATED PRODUCTS

Download our Sample Preparation and Protein Purification Handbooks.





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