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Protocol status: Working

Surface protein biotinylation

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ABSTRACT

This protocol describes surface protein labeling with biotin using EZ-Link Sulfo-NHSLC-Biotin. This chemical reacts with primary amines such as lysine but does not permeate cell membranes because of the charge. Thus, it only biotinylates surface proteins.

ATTACHMENTS

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MATERIALS

Reagents

1. EZ-Link Sulfo-NHS-LC-Biotin

[M] 0.25 mg/mL EZ-Link Sulfo-NHS-LC-Biotin (Thermo) in ice-cold PBS. This solution should be made fresh just before use.

2. Quenching solution

[M] 50 millimolar (mM) glycine in ice-cold PBS (keep at 4 °C).

3. 1% triton X-100 lysis buffer

	A	B
	Tris-HCl, pH 8	20 mM
	Triton X-100	1%
	Glycerol	10%
	NaCl	137 mM
	EDTA	2 mM

Keep at 4 °C and add protease inhibitor cocktails just before use.

4. 2x sample buffer

[M] 100 millimolar (mM) Tris, 4% SDS, 0.2% bromophenol blue, 20% glycerol in DW.

Surface protein biotinylation

5h 5m

1 Wash cells with ice-cold PBS.

Note

Note1. There are many washing steps. Thus, cells may detach from the dishes. If this occurs, coat dishes with poly-D-lysine.

1.1 Wash cells with ice-cold PBS. (1/3)

1.2 Wash cells with ice-cold PBS. (2/3)

1.3 Wash cells with ice-cold PBS. (3/3)

2 Incubate cells with EZ-Link Sulfo-NHS-Biotin for 00:30:00 at 4 °C to label surface proteins. 30m

Note

A rocking platform is recommended.

3 Discard biotin containing medium. Quench and remove unbound biotin using [M] 50 millimolar (mM) glycine in ice-cold PBS for 00:10:00 at 4 °C. 10m

4 Wash 2-3 times with ice-cold PBS.

4.1 Wash with ice-cold PBS. (1/3)

4.2 Wash with ice-cold PBS. (2/3)

4.3 Wash with ice-cold PBS. (3/3)

5 Lysis cells with 1% triton X-100 lysis buffer and centrifuge the samples at 14000 x g, 4°C, 00:20:00 . 20m

6 Collect supernatants and discard the pellets.

7 Measure the protein concentrations using BCA Protein Assay Kit.

8 Incubate the same amount of lysates (500 µg to 1000 µg) with streptavidin or NeutraAvidin beads for 02:00:00 to Overnight at 4 °C to pull-down the biotinylated proteins. 4h

9 Wash the beads with lysis buffer by cycles of suspension.

9.1 Wash the beads with lysis buffer by cycles of suspension. (1/3)

9.2 Wash the beads with lysis buffer by cycles of suspension. (2/3)

9.3 Wash the beads with lysis buffer by cycles of suspension. (3/3)

10 Centrifugation and elute proteins from the packed beads by adding an equal volume of 2x sample buffer and boiling for 00:05:00 95 °C . 5m

11 Run eluate samples on a SDS polyacrylamide gel and perform western blotting to visualize labeling (and thus evidence of surface expression) of the protein of interest. Total cell lysates can be used to determine the expression level.