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Cell surface biotinylation

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Protocol status: Working
We use this protocol and it's working

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Abstract

The protocol describes cell surface biotinylation to identify plasma membrane localized proteins in cell culture via western blotting.

1 grown cells until 70-80% confluency in 10cm dish

2 place cells on ice and wash with ice-cold PBS

3 incubate cells for 30 min c on ice with PBS containing 2.5 mg/ml Sulfo-NHS-SS-biotin (Pierce).

4 stop the biotinylation reaction by washing 3 times for 5min with quenching solution (0.5% BSA and 100 mM glycine in PBS).

5 collect cells by scraping in PBS and centrifugation 500 rpm, 4°C, 00:05:00 5m

6 lyse cells by resuspending cell pellet RIPA buffer supplemented with protease inhibitor and incubating for 30min on ice

7 clear cell lysate by centrifugation (20min, 14000 gavg, 4°C) and collect supernatant

8 isolate biotinylated proteins with immobilized Neutravidin beads

8.1 wash neutravidin beads 2 times with RIPA buffer

8.2 add supernatant from step 7 to beads

8.3 incubate while rotating head-over-head 02:00:00 at 4° 2h

8.4 wash neutravidin beads 3 times with RIPA buffer

9 Input and bound proteins were processed for Western blotting with 4x LDS loading buffer.

9.1 remove all RIPA buffer from neutravidin beads

9.2 add 4x LDS loading buffer

9.3 denature proteins for 10min, 70°C

10 proceed with Western blotting