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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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ASAP

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