

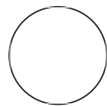


JUL 28, 2023

Cell surface biotinylation

rosanne.wouters¹, Peter Vangheluwe¹

¹KU Leuven



rosanne.wouters

ABSTRACT

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

OPEN  ACCESS



DOI:

dx.doi.org/10.17504/protocols.io.x54v9d9o4g3e/v1

Protocol Citation: rosanne.wouters, Peter Vangheluwe 2023. Cell surface biotinylation. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.x54v9d9o4g3e/v1>



License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Jun 01, 2023


Last Modified: Jul 28, 2023

Keywords: ASAPCRN

- 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 
- 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