JAN 18, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.4r3l27353g1y/v1

Protocol Citation: Cesare Orlandi 2023. Immunoprecipitation from transfected cells. **protocols.io** https://dx.doi.org/10.17504/protocols.io.4r3l27353g1y/v1

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

Created: Jan 18, 2023

Last Modified: Jan 18, 2023

PROTOCOL integer ID:
75491

Immunoprecipitation from transfected cells

Cesare Orlandi[1]

[1]University of Rochester

Cesare Orlandi

ABSTRACT

This protocol provides step by step information on how to perform immunoprecipitation from transfected cells. We normally use this protocol with HEK293FT cells but it can be adopted to other cell types.

GUIDELINES

Note: *All solutions should be ice cold and procedures should be carried out at* ***4°C*** *or* ***on ice****.*

MATERIALS

IP buffer:

Substances	Final conc.	(/50 ml)
H_2O (ice-cold)	N/A	38.25 ml
1 M Tris-HCl (pH 7.4 at 4°C)	50 mM	2.5 ml
4 M NaCl	300 mM	3.75 ml
10% (w/v) Triton X-100	1%	5 ml
add fresh Protease Inhibitors		

Immunoprecipitation from transfected cells

1 Drain medium and gently wash cells once with ice-cold PBS (0.5ml for mw6; 1 ml for 6-cm dish; 3 ml for 10-cm dish).

2 Use a gentle stream to dislodge cells with PBS.

3 Centrifuge the tube at 3000 x g for 1 min and discard the supernatant.

4 Resuspend pellet of cells with 500 µl of ice-cold IP buffer

5 Lyse the cells by sonication: 3 times 10''/30% on ice, waiting 1 minute in between for the lysate to cool down

6 Incubate the whole cell lysate on an end-to-end rocker at 4°C/30' to solubilize the membrane proteins.

7 Spin down at 14,000 x g for 10 minutes at 4°C to pellet debris and genomic DNA, save the supernatant.

8 Save 75 µl, Cell Lysate (CL) and add 25 µl of SB4X

9 Mix total proteins (equal amounts for each sample) with 10-20 µl of Protein G beads and 2 µg of specific antibody. Note: wash Protein G Beads 3 times with PBS to remove ethanol present in the storage buffer. Let equilibrate in IP buffer for minute before use.

10 Incubate on an end-to-end rocker at 4°C/overnight

11 Separate the beads and save 75 µl of Flow Through (FT) and add 2 µl of SB4X

12 10. Wash beads 3 times with 1 ml of IP buffer

13 11. Elute with 50 µl of SB1X (IP)

14 12. Warm samples at 42°C/10’ (membrane proteins would start to aggregate at higher temperature).

15 13. Load 10 µl of each sample per well for SDS-PAGE