Aug 29, 2024

Immunoprecipitation

DOI
dx.doi.org/10.17504/protocols.io.kxygxynzwl8j/v1

Elias Adriaenssens[1]

[1]Sascha Martens lab, University of Vienna, Max Perutz Labs - Vienna

Elias Adriaenssens
Sascha Martens lab, University of Vienna, Max Perutz Labs - ...

DOI: dx.doi.org/10.17504/protocols.io.kxygxynzwl8j/v1

Protocol Citation: Elias Adriaenssens 2024. Immunoprecipitation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kxygxynzwl8j/v1

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

Created: June 21, 2024

Last Modified: August 29, 2024

Protocol Integer ID: 102632

Keywords: ASAPCRN

Funders Acknowledgement:
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP-000350
Marie Skłodowska-Curie MSCA Postdoctoral fellowship
Grant ID: 101062916

Abstract

This protocol details the process of immunoprecipitation.

Materials

- Pierce™ Detergent Compatible Bradford Assay Kit **Thermo Fisher Catalog #**23246
- NuPAGE™ 4-12% Bis-Tris Protein Gels, 1.0 mm, 12-well **Thermo Fisher Catalog #**NP0322BOX
  Pageruler Prestained Protein Ladder **Thermo Fisher Scientific Catalog #**26616
- nitrocellulose membranes (RPN132D, GE Healthcare)
- Mini Trans-Blot Cell (Bio-Rad)
- SuperSignal West Femto Maximum Sensitivity Substrate (34096, Thermo Fisher)
  SuperSignal™ West Femto Maximum Sensitivity Substrate **Thermo Fisher Catalog #**34096
- ChemiDoc MP Imaging system (Bio-Rad).

Lysis buffer:

KCl	100 mM
MgCl2	2.5 mM
Tris-HCl	20 mM
pH	7.4
NP-40	0.50%

Steps

30m

1 Collect the HeLa cells by trypsinization and wash the cell pellet with PBS once before cells are lysed in lysis buffer (100 mM KCl, 2.5 mM $MgCl_2$, 20 mM Tris-HCl pH 7.4, 0.5% NP-40).

KCl	100 mM
MgCl2	2.5 mM
Tris-HCl	20 mM
pH	7.4
NP-40	0.50%

2 Lyse the samples for 00:20:00 On ice before cell lysates are cleared by centrifugation at 20000 x g, 4°C, 00:10:00 .

30m

3 Protein concentrations of the cleared protein lysates are then determined with the Pierce Detergent Compatible Bradford Assay Kit (23246, Thermo Fisher) and equal amounts are incubated with beads.

4 Precoat the beads with GST (negative control), NIX-GST, or BNIP3-GST as described in the protocol for the microscopy-based bead assay.

5 Incubate the HeLa cell lysates Overnight with precoated beads.

20m

6 In the morning, wash the samples five times in lysis buffer before the beads are either submitted for analysis by mass spectrometry or for analysis by SDS-PAGE and western blotting by resuspending the beads in protein loading dye, supplemented with [M] 100 millimolar (mM) DTT, and boiled for 00:05:00 at 95 °C .

5m

Note that for mass spec samples, the NP-40 is omitted from the washing buffer.

7 Load the samples on 4-12% SDS-PAGE gels (NP0322BOX, Thermo Fisher) with PageRuler Prestained protein marker (Thermo Fisher).

8 Transfer the proteins onto nitrocellulose membranes (RPN132D, GE Healthcare) for 01:00:00 at 4 °C using the Mini Trans-Blot Cell (Bio-Rad).

1h

9 After the transfer, membranes are blocked with 5% milk powder dissolved in PBS-Tween (0.1% Tween 20) for 01:00:00 at Room temperature . 1h

10 Incubate the membranes Overnight at 4 °C with primary antibodies dissolved in the blocking buffer, wash three times for 5 min, and incubate with species-matched secondary horseradish peroxidase (HRP)-coupled antibodies diluted 1:10,000 in blocking buffer for 01:00:00 at Room temperature . 2h

10.1 Wash for 00:05:00 (1/3). 5m

10.2 Wash for 00:05:00 (2/3). 5m

10.3 Wash for 00:05:00 (3/3). 5m

11 Wash the membranes three times with PBS-T and processed further for western blot detection.

12 Incubate the membranes with SuperSignal West Femto Maximum Sensitivity Substrate (34096, Thermo Fisher) and imaged with a ChemiDoc MP Imaging system (Bio-Rad).

13 Analyze the images with ImageJ 47 (RRID:SCR_003070; https://imagej.net/).