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Immunoprecipitation

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We use this protocol and it's working

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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
	MgCl ₂	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 MgCl₂, 20 mM Tris-HCl pH 7.4, 0.5% NP-40).



	KCl	100 mM
	MgCl ₂	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 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/>).