Mar 21, 2025

Co-immunoprecipitation V5-NIX

Nature Cell Biology

DOI
https://dx.doi.org/10.17504/protocols.io.q26g7m6qqgwz/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: https://dx.doi.org/10.17504/protocols.io.q26g7m6qqgwz/v1

External link: https://doi.org/10.1038/s41556-025-01712-y

Protocol Citation: Elias Adriaenssens 2025. Co-immunoprecipitation V5-NIX. **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g7m6qqgwz/v1

Manuscript citation:
Adriaenssens, E., Schaar, S., Cook, A.S.I. *et al.* Reconstitution of BNIP3/NIX-mitophagy initiation reveals hierarchical flexibility of the autophagy machinery. *Nat Cell Biol* **27**, 1272–1287 (2025). https://doi.org/10.1038/s41556-025-01712-y

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

Created: January 21, 2025

Last Modified: March 21, 2025

Protocol Integer ID: 119231

Keywords: ASAPCRN, protocol detail, protocol

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

Abstract

This protocol details co-immunoprecipitation.

Materials

Materials and Reagents

Lysis Buffer:

	A	B
	KCl	150 mM
	MgCl2	2.5 mM
	Tris-HCl pH 7.4	20 mM
	NP-40	0.50%

Washing Buffer:

	A	B
	KCl	150 mM
	MgCl2	2.5 mM
	Tris-HCl pH 7.4	20 mM

- V5-NIX (RRID:Addgene_223731)
- Anti-V5 agarose beads **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A7345
- 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

Co-immunoprecipitation

1h

1 Stably transduce BNIP3/NIX double knockout HeLa cells with lentivirus to express V5-NIX (RRID:Addgene_223731).

2 After seeding, treat the cells with DFP to induce mitophagy, where indicated.

3 After the treatment, collect the cells by trypsinization and wash the cell pellet with PBS once before cells were lysed in lysis buffer.

Lysis buffer:

	A	B
	KCl	150 mM
	$MgCl_2$	2.5 mM
	Tris-HCl pH 7.4	20 mM
	NP-40	0.50%

4 Lyse samples for 00:20:00 On ice . Then, centrifuge the cell lysates at 20000 x g, 4°C, 00:10:00 .

30m

5 Then, determine the protein concentrations of the cleared protein lysates with the Pierce Detergent Compatible Bradford Assay Kit (23246, Thermo Fisher).

6 Incubate 7 mg of cell lysate Overnight with 20 µL of anti-V5 agarose beads (A7345, Sigma-Aldrich).

20m

7 In the morning, wash the samples three times in washing buffer and resuspend in protein loading dye, supplement it with [M] 100 millimolar (mM) DTT, and boil it for 00:05:00 at 95 °C .

5m

Washing buffer:

	A	B
	KCl	150 mM
	$MgCl_2$	2.5 mM
	Tris-HCl pH 7.4	20 mM

8 Load the samples on 4-12% SDS-PAGE gels (NP0322BOX, Thermo Fisher) and analyze them by western blotting as described above.