

Aug 29, 2024

Purification of GST-WIPI1/WIPI2d/WIPI3/WIPI4

 [Nature Cell Biology](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.n2bvjnnqxgk5/v1>

Elias Adriaenssens¹

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



Elias Adriaenssens

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

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.n2bvjnnqxgk5/v1>

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

Protocol Citation: Elias Adriaenssens 2024. Purification of GST-WIPI1/WIPI2d/WIPI3/WIPI4. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.n2bvjnnqxgk5/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>

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: May 24, 2024

Last Modified: August 29, 2024

Protocol Integer ID: 101115

Keywords: ASAPCRN, wipi4 this protocol, purification of gst, wipi2d, protocol details the purification, wipi3, wipi4, wipi1, purification, gst, protocol detail

Funders Acknowledgements:

Aligning Science Across Parkinson's (ASAP)

Grant ID: ASAP-000350

Marie Skłodowska-Curie MSCA Postdoctoral fellowship

Grant ID: 101062916

Abstract

This protocol details the purification of GST-tagged WIP1/2d/3/4.

Materials

 FreeStyle™ 293 Expression Medium **Thermo Fisher Scientific Catalog #12338026**

 Opti-MEM™ I Reduced Serum Medium **Thermo Fisher Scientific Catalog #31985062**

 Polyethylenimine, Linear, MW 25000, Transfection Grade (PEI 25K™) **Polysciences, Inc. Catalog #23966-1**

 EX-CELL® 293 Serum-Free Medium for HEK 293 Cells **Merck MilliporeSigma (Sigma-Aldrich) Catalog #14571C**

25ml lysis buffer:

	A	B
	Tris-HCl, pH 7.4	50 mM
	NaCl	300 mM
	MgCl ₂	2 mM
	Glycerol	5%
	Triton X-100	1%
	β-mercaptoethanol	2 mM

Wash buffer:

	A	B
	Tris-HCl, pH 7.4	50 mM
	NaCl	300 mM
	DTT	1 mM

Salt wash buffer:

	A	B
	Tris-HCl, pH 7.4	50 mM
	NaCl	700 mM
	DTT	1 mM

SEC buffer:



	A	B
	Tris-HCl, pH 7.4	25 mM
	NaCl	150 mM
	DTT	1 mM



Purification procedure

1d 5h 5m 30s

- 1 To purify GST-WIP1/GST-WIP2/GST-WIP3/GST-WIP4, we express the GST-tagged WIP1/2d/3/4 from a pCAG backbone encoding GST-TEV-WIP1/2/3/4 (available from Addgene).
- 2 Express the protein in FreeStyle™ HEK293F cells, grown at 37 °C in FreeStyle™ 293 Expression Medium (Thermo, 12338-026).
- 3 The day before transfection, seed the cells at a density of 0.7×10^6 cells per ml.
- 4 On the day of transfection, transfect a 400 mL culture with 400 µL of the MAXI-prep DNA, diluted in 13 mL of Opti-MEMR I Reduced Serum Medium (Thermo, 31985-062), and 800 µL Polyethylenimine (PEI 25K, Polysciences CatNo 23966-1), also diluted in 13 mL of Opti-MEM media.
- 5 One day post transfection, supplement the culture with 100 mL EXCELL R 293 Serum-Free Medium (Sigma-Aldrich, 14571C- 1000ML).
- 6 Another 24:00:00 later, harvest the cells by centrifugation at 270 x g, 00:20:00 .
- 7 Wash the pellet with PBS to remove medium and then flash-frozen in liquid nitrogen.
- 8 Store the pellets at -80 °C .
- 9 For purification of GST-TEV-WIP1/2/3/4, resuspend the cell pellet in, (complete EDTA-free protease inhibitors (Roche), CIP protease inhibitor (Sigma), and DNase (Sigma)).

25ml lysis buffer:



	A	B
	Tris-HCl, pH 7.4	50 mM
	NaCl	300 mM
	MgCl ₂	2 mM
	Glycerol	5%
	Triton X-100	1%
	β-mercaptoethanol	2 mM

- 10 Sonicate the cell lysates twice for 00:00:30 . 30s
- 11 Clear the cell lysates by centrifugation at 10000 rpm, 4°C, 00:45:00 with a SORVAL RC6+ centrifuge with an F21S-8×50Y rotor (Thermo Scientific). 45m
- 12 Collect and incubate the supernatant with pre-equilibrated Glutathione Sepharose 4B beads (GE Healthcare) for 02:00:00 at 4 °C with gentle shaking to bind GST-TEV-WIP1/2/3/4. 2h
- 13 Centrifuge the samples to pellet the beads and remove the unbound lysate.
- 14 Wash the beads twice with wash buffer, once with high salt wash buffer, and two more times with wash buffer.

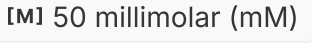
Wash buffer:

	A	B
	Tris-HCl, pH 7.4	50 mM
	NaCl	300 mM
	DTT	1 mM

Salt wash buffer:



	A	B
	Tris-HCl, pH 7.4	50 mM
	NaCl	700 mM
	DTT	1 mM

- 15 Incubate the beads  Overnight with  4 mL of  50 millimolar (mM) reduced glutathione dissolved in wash buffer at  4 °C , to elute GST-tagged WIP1/2/3/4 from the beads.

2h

**Wash buffer:**

	A	B
	Tris-HCl, pH 7.4	50 mM
	NaCl	300 mM
	DTT	1 mM

- 16 To collect the supernatant, collect the beads by centrifugation.

- 17 Wash the beads twice with  4 mL of wash buffer, and collect the supernatant.



- 18 Pool and filterate the supernatant fractions through a 0.45 µm syringe filter, concentrate with 30 kDa cut-off Amicon filter (Merck Millipore), and load onto a pre-equilibrated Superdex 200 Increase 10/300 GL column (Cytiva).

- 19 Elute the proteins with SEC buffer.

SEC buffer:

	A	B
	Tris-HCl, pH 7.4	25 mM
	NaCl	150 mM
	DTT	1 mM



20 Analyse the fractions by SDS-PAGE and Coomassie staining.



21 Pool the fractions containing purified GST-TEV-WIP1/2/3/4.

22 After concentrating the purified protein, aliquot the protein and snap-frozen in liquid nitrogen.

23 Store the proteins at  -80 °C .