



Aug 29, 2024

Purification of mCh-WIPI2d-IDR (364-426aa)

 [Nature Cell Biology](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.5qpvokk8bl4o/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.5qpvokk8bl4o/v1>

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

Protocol Citation: Elias Adriaenssens 2024. Purification of mCh-WIPI2d-IDR (364-426aa). **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.5qpvokk8bl4o/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: 101116

Keywords: ASAPCRN, purification of mcherry wipi2d, purification of mcherry, mcherry wipi2d, protocol details the purification, purification, wipi2d, idr, mcherry, 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 mCherry WIP12d-IDR.

Materials

 Rosetta™(DE3)pLysS Competent Cells - Novagen **Merck Catalog #70956-4**

Lysis buffer:

	A	B
	Tris-HCl, pH 7.4	50 mM
	NaCl	300 mM
	MgCl ₂	2 mM
	Glycerol	5%
	Triton X-100	1%
	Imidazole	10 mM
	β-mercaptoethanol	2 mM

Wash buffer:

	A	B
	Tris-HCl, pH 7.4	50 mM
	NaCl	300 mM
	Imidazole	10 mM
	β-mercaptoethanol	2 mM

SEC buffer:

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



Purification procedure

16h 45m 30s

- 1 To purify mCherry-WIPI2d IDR (364-426aa) (available from Addgene), fuse the corresponding coding sequence of WIPI2d to a N-terminal 6xHis-TEV-mCherry-tag through cloning into a pET-DUET1 vector (available from Addgene).
- 2 After the transformation of the pET-DUET1 vector encoding 6xHis-TEV-mCherry-WIPI2d-IDR in *E. coli* Rosetta pLysS cells (Novagen Cat# 70956-4), grow cells in 2x Tryptone Yeast extract (TY) medium at  37 °C until an OD₆₀₀ of 0.4 and then continued at  18 °C .
- 3 Once the cells reach an OD₆₀₀ of 0.8, induce the protein expression with  100 micromolar (μM) isopropyl β-D-1-thiogalactopyranoside (IPTG) for  16:00:00 at  18 °C .
- 4 Collect the cells by centrifugation and resuspend in lysis buffer, complete EDTA-free protease inhibitors (Roche), CIP protease inhibitor (Sigma), and DNase (Sigma)).

16h

Lysis buffer:

A	B
Tris-HCl, pH 7.4	50 mM
NaCl	300 mM
MgCl ₂	2 mM
Glycerol	5%
Triton X-100	1%
Imidazole	10 mM
β-mercaptoethanol	2 mM

- 5 Sonicate the cell lysates twice for  00:00:30 .
- 6 Clear the lysates by centrifugation at  18000 rpm, 4°C,  00:45:00 in a SORVAL RC6+ centrifuge with an F21S-8×50Y rotor (Thermo Scientific).

30s

45m



- 7 Filter the supernatant through an 0.45 µm filter and load onto a pre-equilibrated 5 ml His-Trap HP column (Cytiva).
- 8 After bind His tagged proteins to the column, was the column with three column volumes of wash buffer.

Wash buffer:

	A	B
	Tris-HCl, pH 7.4	50 mM
	NaCl	300 mM
	Imidazole	10 mM
	β-mercaptoethanol	2 mM

- 9 Elute the proteins with a stepwise imidazole gradient (30, 75, 100, 150, 225, 300 mM).
- 10 Pool fractions containing the 6xHis-TEV-mCherry-WIPI2d-IDR, concentrate using a 10 kDa cut-off Amicon filter (Merck Millipore) and load onto a pre-equilibrated S75 Increase 10/300 column (Cytiva).
- 11 Elute the proteins with SEC buffer.

SEC buffer:

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

- 12 Analyse the fractions by SDS-PAGE and Coomassie staining.
- 13 Pool the fractions containing purified mCherry-WIPI2d-IDR.





- 14 After concentrating the purified protein, aliquot the protein and snap-frozen in liquid nitrogen.
- 15 Store the proteins at  -80 °C .