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Purification of GST-WIPI1/WIPI2d/WIPI3/WIPI4

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We use this protocol and it's working

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Abstract

This protocol details the purification of GST-tagged WIPI1/2d/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
	MgCl2	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-WIPI1/GST-WIPI2/GST-WIPI3/GST-WIPI4, we express the GST-tagged WIPI1/2d/3/4 from a pCAG backbone encoding GST-TEV-WIPI1/2/3/4 (available from Addgene).

2 Express the protein in FreeStyleTM 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 x 10^6 cells per ml.

4 On the day of transfection, transfect a 400 mL culture with 400 undetermined of the MAXI-prep DNA, diluted in 13 mL of Opti-MEMR I Reduced Serum Medium (Thermo, 31985-062), and 800 undetermined 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 . 1d 0h 20m

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-WIPI1/2/3/4, 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

	A	B
	NaCl	300 mM
	MgCl2	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-8x50Y 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-WIPI1/2/3/4. 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 [M] 50 millimolar (mM) reduced glutathione dissolved in wash buffer at 4 °C, to elute GST-tagged WIPI1/2/3/4 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-WIPI1/2/3/4.

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

23 Store the proteins at -80 °C .