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🌐 Expression and purification protocol of WIPI2d

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ABSTRACT

This protocol details the expression and purification protocol of WIPI2d.

ATTACHMENTS

[246-486.docx](#)

MATERIALS

General information:

A	B
Expression system	Human embryonic kidney (HEK) 293 GnTI
Medium	Freestyle media + Anti-Anti
Plasmid origin	Addgene 171419
Backbone	pCAG
Resistance	Amp
Insert	Homo sapiens WIPI2d (NP_001028691.1)
Tags & cleavage sites	C-term TEV-TwinStrep-Flag (TSF)
Ext coeff	37110 M-1cm-1, MW 50.7 kDa

Lysis Buffer:

A	B
Hepes pH=7.4	50 mM
Triton X-100	1%
NaCl	200 mM
MgCl ₂	1 mM
Glycerol	10%

OPEN ACCESS

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

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A	B
TCEP	1 mM
EDTA-free Protease Inhibitors (Roche)	

Wash Buffer:

A	B
Hepes pH=7.5	50 mM
NaCl	200 mM
TCEP	1 mM

Elution Buffer:

A	B
Hepes pH=7.5	50 mM
NaCl	200 mM
TCEP	1 mM
Desthiobiotin	10 mM

SEC Buffer:

A	B
Hepes pH=8	20 mM
NaCl	150 mM
TCEP	1 mM

Columns/Resin:

- Glutathione Sepharose 4B
- S200_16/60 prep grade, GE Healthcare

Protein expression

- 1 Transfect the DNA in cells using polyethylenimine (Polysciences).

- 2 After 48- to 72-hour expression, harvest the cells.

Protein purification

4h

- 3 Follow the Strep-Tactin batch purification by Size Exclusion Chromatography.

- 4 Resuspend the cell pellet in Lysis Buffer and clear at 16000 rpm at 4 °C for 01:00:00 .

1h

- 5 Incubate the supernatant with Strep-Tactin Sepharose resin at 4 °C with gentle shaking for 03:00:00 , apply to a gravity column, and wash extensively with Wash Buffer.

3h

- 6 Elute the protein of interest with Elution Buffer and then apply onto a Superdex 200 column (16/60 prep grade) pre-equilibrated in SEC Buffer at 4 °C .

- 7 Pool and concentrate the peak fractions containing pure protein, snap-frozen in liquid nitrogen and store at -80 °C .

Note

Yield: about 2.5 mg per liter culture