Expression and purification protocol of WIPI2d

Chunmei Chang[1]

[1]James Hurley Lab, UC, Berkeley

Jun 12, 2021

1 Works for me Share dx.doi.org/10.17504/protocols.io.bvjnn4me

Chunmei Chang

ABSTRACT

This protocol details the expression and purification protocol of WIPI2d.

ATTACHMENTS

246-486.docx

DOI

dx.doi.org/10.17504/protocols.io.bvjnn4me

PROTOCOL CITATION

Chunmei Chang 2021. Expression and purification protocol of WIPI2d. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bvjnn4me

CREATED

Jun 06, 2021

LAST MODIFIED

Jun 12, 2021

OWNERSHIP HISTORY

Jun 06, 2021 Urmilas

Jun 11, 2021 Chunmei Chang

PROTOCOL INTEGER ID

50510

MATERIALS TEXT

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
MgCl2	1 mM
Glycerol	10%
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).

Citation: Chunmei Chang (06/12/2021). Expression and purification protocol of WIPI2d. https://dx.doi.org/10.17504/protocols.io.bvjnn4me

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 1h

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

5 3h

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.

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** .

Yield: about 2.5 mg per liter culture

Citation: Chunmei Chang (06/12/2021). Expression and purification protocol of WIPI2d. https://dx.doi.org/10.17504/protocols.io.bvjnn4me