May 25, 2022

Expression and purification protocol of GST-mCh-FYVE

Expression and purification protocol of GST-NDP52

Liv Jensen[1], Chunmei Chang[2]

[1]James Hurley Lab, University of California Berkeley; [2]James Hurley Lab, UC, Berkeley

1

dx.doi.org/10.17504/protocols.io.ewov1n39pgr2/v1

Liv Jensen

This protocol details the expression and purification of GST-mCherry-FYVE.

246-484.docx

DOI

dx.doi.org/10.17504/protocols.io.ewov1n39pgr2/v1

Liv Jensen, Chunmei Chang 2022. Expression and purification protocol of GST-mCh-FYVE. **protocols.io** https://dx.doi.org/10.17504/protocols.io.ewov1n39pgr2/v1

Expression and purification protocol of GST-NDP52, Chunmei Chang

ASAPCRN

protocol ,

May 02, 2022

May 25, 2022

61821

General information:

A	B
Expression system	E.Coli BL21DE3
Medium	Luria Bertani
Plasmid origin	
Backbone	pGST2
Resistance	Amp
Insert	
Tags & cleavage sites	N-term GST
Ext coeff	82320 M-1cm-1, MW 77.9 kDa

Lysis Buffer:

A	B
Hepes pH=7.5	50 mM
NaCl	300 mM
TCEP	1 mM
Protease Inhibitors (Roche)	

Wash Buffer:

A	B
Hepes pH=7.5	50 mM
NaCl	150 mM
TCEP	1 mM

Elution Buffer:

A	B
Hepes pH=7.5	50 mM
NaCl	150 mM
TCEP	1 mM
Glutathione	50 mM

SEC Buffer:

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

Columns/Resin:

- Glutathione Sepharose 4B

Citation: Liv Jensen, Chunmei Chang Expression and purification protocol of GST-mCh-FYVE
https://dx.doi.org/10.17504/protocols.io.ewov1n39pgr2/v1

- S6_10/300 Increase

Protein expression 20m

1 Transform the E.Coli BL21DE3 cells with plasmid encoding for GST-mCh-FYVE and plate them on Amp plate.

2 

Carry out protein expression in **1 L** medium, induce with **200 micromolar (µM)** IPTG (isopropyl- β-d-thiogalactopyranoside) to an OD_{600} of 0.8 and grow at **18 °C** **Overnight** .

3 

20m

Harvest the cells by spinning at **4500 x g** for **00:20:00** at **4 °C** and stock at **-80 °C** until purification.

Protein purification 5h

4 Follow the GST batch purification by Size Exclusion Chromatography.

5 Resuspend the pellets in Lysis Buffer, sonicate for cell lysis and clear at **16000 rpm** at **4 °C** for **01:00:00**

1h

6 

30m

Incubate the supernatant with Glutathione Sepharose 4B (GE Healthcare) at **4 °C** with gentle shaking for **00:30:00** , apply to a gravity column, and wash extensively with Wash Buffer.

7 Elute the protein of interest with Elution Buffer and then apply onto a Superdex 6 column (10/300 Increase) pre-equilibrated in SEC Buffer at **4 °C** .

Citation: Liv Jensen, Chunmei Chang Expression and purification protocol of GST-mCh-FYVE
https://dx.doi.org/10.17504/protocols.io.ewov1n39pgr2/v1

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

Citation: Liv Jensen, Chunmei Chang Expression and purification protocol of GST-mCh-FYVE
https://dx.doi.org/10.17504/protocols.io.ewov1n39pgr2/v1