Expression and purification protocol of GST-TAX1BP1

Chunmei Chang[1]
[1]James Hurley Lab, UC, Berkeley

Jun 12, 2021

dx.doi.org/10.17504/protocols.io.bvjen4je

Chunmei Chang

ABSTRACT

This protocol details the expression and purification protocol of GST-TAX1BP1.

ATTACHMENTS

246-485.docx

DOI

dx.doi.org/10.17504/protocols.io.bvjen4je

PROTOCOL CITATION

Chunmei Chang 2021. Expression and purification protocol of GST-TAX1BP1. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bvjen4je

KEYWORDS

GST-TAX1BP1, Protein expression, Protein purification

CREATED

Jun 05, 2021

LAST MODIFIED

Jun 12, 2021

OWNERSHIP HISTORY

Jun 05, 2021 Urmilas

Jun 11, 2021 Chunmei Chang

PROTOCOL INTEGER ID

50502

MATERIALS TEXT

General information:

A	B
Expression system	E.Coli BL21DE3
Medium	Luria Bertani
Plasmid origin	Addgene 171423
Backbone	pGST2
Resistance	Amp
Insert	Homo sapiens (NM_006024.7)
Tags & cleavage sites	N-term GST
Ext coeff	103020 M-1cm-1, MW 116.6 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	300 mM
TCEP	1 mM

Elution Buffer:

A	B
Hepes pH=7.5	50 mM
NaCl	300 mM
TCEP	1 mM

SEC Buffer:

A	B
Hepes pH=8	20 mM
NaCl	200 mM
TCEP	1 mM

Columns/Resin:

- Glutathione Sepharose 4B
- S6_10/300 Increase

Protein expression 20m

1 Transform the E.Coli BL21DE3 cells with a plasmid encoding for GST-TAX1BP1 and plate them on Amp plate.

2

Citation: Chunmei Chang (06/12/2021). Expression and purification protocol of GST-TAX1BP1. https://dx.doi.org/10.17504/protocols.io.bvjen4je

Carry out protein expression in **1.5 L** LB medium, induce with **100 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 4h

Incubate the supernatant with Glutathione Sepharose 4B (GE Healthcare) at **4 °C** with gentle shaking for **04:00:00** , 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** .

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

Citation: Chunmei Chang (06/12/2021). Expression and purification protocol of GST-TAX1BP1. https://dx.doi.org/10.17504/protocols.io.bvjen4je