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Expression and purification protocol of GST-TAX1BP1

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1	Works for me	≪ Sha	are	dx.doi.org/10.17504/protocols.io.bvjen4je

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ABSTRAC1

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

ATTACHMENTS

246-485.docx

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

PROTOCOL CITATION

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KEYWORDS

GST-TAX1BP1, Protein expression, Protein purification

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OWNERSHIP HISTORY

Jun 05, 2021 Urmilas

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PROTOCOL INTEGER ID

50502

MATERIALS TEXT

General information:

A	В
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:

Α	В
Hepes pH=7.5	50 mM
NaCl	300 mM
TCEP	1 mM
Protease Inhibitors (Roche)	

Wash Buffer:

Α	В
Hepes pH=7.5	50 mM
NaCl	300 mM
TCEP	1 mM

Elution Buffer:

Α	В
Hepes pH=7.5	50 mM
NaCl	300 mM
TCEP	1 mM

SEC Buffer:

Α	В
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.





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2

Carry out protein expression in \blacksquare **1.5** L LB medium, induce with [M]**100 Micromolar (\muM)** IPTG (isopropyl- β -d-thiogalactopyranoside) to an OD₆₀₀ of 0.8 and grow at \$ **18 °C** \bigcirc **Overnight**.

3

20m

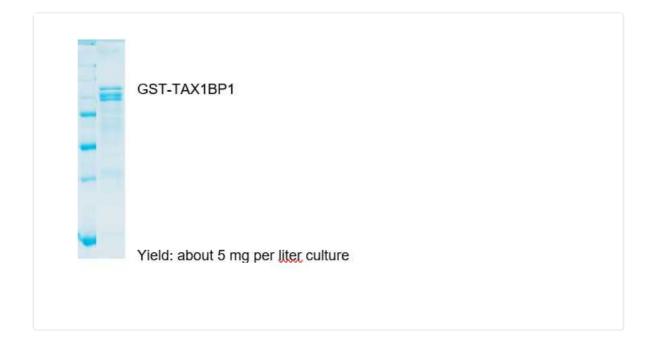
Harvest the cells by spinning at **§34500 x g** for **© 00:20:00** at **§4 °C** and stock at **§ -80 °C** until purification.

Protein purification

- 4 Follow the GST batch purification by Size Exclusion Chromatography.
- Resuspend the pellets in Lysis Buffer, sonicate for cell lysis and clear at **16000 rpm** at **84°C** for **001:00:00**
- 6 🗇 🙈

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) preequilibrated in SEC Buffer at § 4 °C.
- 8 Pool the peak fractions containing pure protein, snap-frozen in liquid nitrogen and store at 8-80 °C.



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