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Expression and purification protocol of GST-OPTN or (S177D, S473D)

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ABSTRACT

This protocol details the expression and purification protocol of GST-OPTN or (S177D, S473D).

ATTACHMENTS

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PROTOCOL CITATION

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KEYWORDS

GST-OPTN, S177D, S473D, Protein expression, Protein purification

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MATERIALS TEXT

General information:

A	B
Expression system	E.Coli BL21DE3
Medium	Luria Bertani
Plasmid origin	Addgene 171424 or 171425
Backbone	pGST2
Resistance	Amp
Insert	Homo sapiens (NM_001008212.2)
Tags & cleavage sites	N-term GST
Ext coeff	59060 M-1cm-1, MW 91.6 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 plasmid encoding for GST-OPTN and plated them on Amp plate.

2

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Carry out the 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 1h

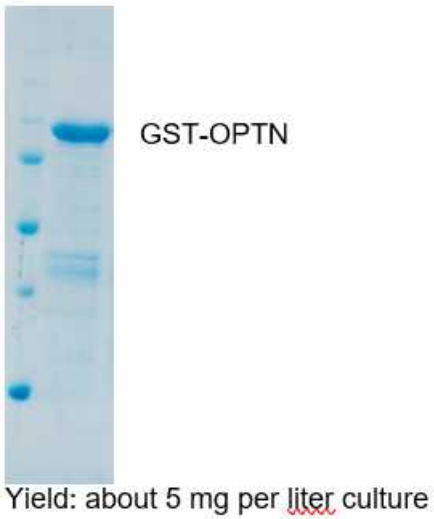
Resuspend the pellets in Lysis Buffer, sonicate for cell lysis and clear at **16000 rpm** at **4 °C** for **01:00:00** .

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** .



Yield: about 5 mg per liter culture

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