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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

[247-488.docx](#)

MATERIALS

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 ⁻¹ cm ⁻¹ , MW 91.6 kDa

Lysis Buffer:

A	B
Hepes pH=7.5	50 mM
NaCl	300 mM

OPEN ACCESS

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protocols.io
<https://dx.doi.org/10.17504/protocols.io.bvjrn4m6>

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Protocol status: Working
 We use this protocol and it's working

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 50513

Keywords: GST-OPTN, S177D, S473D, Protein expression, Protein purification, ASAPCRN

A	B
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

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

Harvest the cells by spinning at 4500 x for 00:20:00 at 4 °C and stock at

-80 °C until purification.

20m

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

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.

4h

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 .

Note

GST-OPTN

Yield: about 5 mg per liter culture