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Purification of OPTN-GST

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ABSTRACT

This protocol describes purification of OPTN-GST.

ATTACHMENTS

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MATERIALS

Materials:

- pETDuet-1 vector (Vector encoding OPTN-GST is available from Addgene)
- Glutathione Sepharose 4B beads (GE Healthcare)
- Amicon filter (Merck Millipore)
- Superdex 200 Increase 10/300 GL column (Cytiva)

Lysis buffer:

	A	B
	HEPES pH 7.4	50 mM
	NaCl	300 mM
	MgCl2	2 mM
	Glycerol	5%
	Imidazole	10 mM
	β-mercaptoethanol	2 mM
	cOmplete EDTA-free protease inhibitors (Roche)	
	CIP protease inhibitor (Sigma)	
	DNase (Sigma)	

Wash Buffer:

	A	B
	HEPES pH 7.4	50 mM
	NaCl	300 mM
	DTT	1 mM

High-salt wash buffer:

	A	B
	HEPES pH 7.4	50 mM
	NaCl	700 mM
	DTT	1 mM

SEC buffer:

	A	B
	HEPES pH 7.4	25 mM
	NaCl	150 mM
	DTT	1 mM

Equipment

- SORVAL RC6+ centrifuge
- Beckman Ti45 rotor

Purification of OPTN-GST

20h 45m 30s

1 To purify OPTN-GST, Clone human OPTN cDNA in a pETDuet-1 vector with an C-terminal GST-tag.

2 After the transformation of the pETDuet-1 vector encoding OPTN-GST in E. coli Rosetta pLysS cells, grow the cells in 2xTY medium at 37 °C until an OD_{600} of 0.4 and then continue at 18 °C.

3 Once the cells reached an OD_{600} of 0.8, induce protein expression with [M] 50 micromolar (µM) IPTG for 16h 16:00:00 at 18 °C

4 Collect the cells by centrifugation and resuspend in lysis buffer.

Lysis buffer

	A	B
	HEPES pH 7.4	50 mM
	NaCl	300 mM
	MgCl2	2 mM
	Glycerol	5%
	Imidazole	10 mM
	β-mercaptoethanol	2 mM
	cOmplete EDTA-free protease inhibitors (Roche)	
	CIP protease inhibitor (Sigma)	
	DNase (Sigma)	

5 Sonicate cell lysates. 1m

5.1 Sonicate cell lysates twice for 00:00:30 (1/2). 30s

5.2 Sonicate cell lysates twice for 00:00:30 (2/2). 30s

6 Clear the lysates by centrifugation at 18000 rpm, 4°C, 00:45:00 in a SORVAL RC6+ centrifuge with an F21S-8x50Y rotor (Thermo Scientific). 45m

7 Collect and incubate the supernatant with pre-equilibrated Glutathione Sepharose 4B beads (GE Healthcare) for 02:00:00 at 4 °C with gentle shaking to bind OPTN-GST. 2h

8 Centrifuge the samples to pellet the beads and remove the unbound lysate.

9 Wash beads twice with wash buffer, once with high salt wash buffer, and two more times with wash buffer.

Wash buffer

	A	B
	HEPES pH 7.4	50 mM
	NaCl	300 mM
	DTT	1 mM

High-salt wash buffer

	A	B
	HEPES pH 7.4	50 mM
	NaCl	700 mM
	DTT	1 mM

10 Elute the proteins Overnight with [M] 20 millimolar (mM) reduced L-glutathione in [M] 50 millimolar (mM) HEPES pH 7.4, [M] 300 millimolar (mM) NaCl, [M] 1 millimolar (mM) DTT buffer. 2h

11 Collect the supernatant, filter through a 0.45 µm syringe filter, concentrate using a 50 kDa cut-off Amicon filter (Merck Millipore), and load onto a pre-equilibrated Superdex 200 Increase 10/300 GL column (Cytiva).

12 Elute the proteins with SEC buffer.

SEC buffer

	A	B
	HEPES pH 7.4	25 mM
	NaCl	150 mM
	DTT	1 mM

13 Analyze fractions by SDS-PAGE and Coomassie staining.

14 Pool fractions containing purified OPTN-GST.

15 After concentrating the purified protein, aliquot the protein and snap-frozen it in liquid nitrogen. Store proteins at -80 °C.