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Purification of OPTN-GST

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Abstract

This protocol describes purification of OPTN-GST.

Attachments



[pphpb32rp.pdf](#)

27KB



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
	MgCl ₂	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 OD₆₀₀ of 0.4 and then continue at 18 °C .
- 3 Once the cells reached an OD₆₀₀ of 0.8, induce protein expression with 50 micromolar (μM) IPTG for 16:00:00 at 18 °C
- 4 Collect the cells by centrifugation and resuspend in lysis buffer.

16h



Lysis buffer

A	B
HEPES pH 7.4	50 mM
NaCl	300 mM
MgCl ₂	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.
- 5.1 Sonicate cell lysates twice for 00:00:30 (1/2).

1m

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-8×50Y 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  20 millimolar (mM) reduced L-glutathione in  50 millimolar (mM) HEPES  7.4 ,  300 millimolar (mM) NaCl,  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 .