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Purification of FKBP8-GST

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We use this protocol and it's working

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Abstract

This protocol describes the purification and analysis of FKBP8-GST by SDS-PAGE and Coomassie staining.

Materials

Lysis buffer:

	A	B
	Tris-HCl pH 7.4	50 mM
	NaCl	300 mM
	Triton X-100	1%
	Glycerol	5%
	MgCl2	2 mM
	DTT	1 mM
	β-mercaptoethanol	2 mM
	cOmplete EDTA-free protease inhibitors (Roche)	
	CIP protease inhibitor (Sigma)	
	DNase (Sigma)	

Wash Buffer:

	A	B
	Tris-HCl pH 7.4	50 mM
	NaCl	300 mM
	DTT	1 mM

Salt wash Buffer:

	A	B
	Tris-HCl pH 7.4	50 mM
	NaCl	700 mM
	DTT	1 mM

SEC Buffer:

	A	B
	Tris-HCl pH 7.4	25 mM
	NaCl	300 mM
	DTT	1 mM

- Rosetta™(DE3)pLysS Competent Cells - Novagen **Merck Catalog #**70956-4
- pET-Duet encoding FKBP8 (1-391aa)-thrombin-GST (available from Addgene)

Purification procedure

1d 2h 45m 30s

1 To purify FKBP8-GST, fuse the cytosol-exposed domain of FKBP8 (1-391aa) to a C-terminal GST-tag and clone into a pET-DUET1 vector.

2 After the transformation of the pET-DUET1 vector encoding FKBP8-GST in *E.coli* Rosetta pLysS cells (Novagen Cat# 70956-4), grow cells in 2x Tryptone Yeast extract (TY) medium at 37 °C until an OD_{600} of 0.4 and then continue at 18 °C.

3 Once the cells reach an OD_{600} of 0.8, induce the protein expression with [M] 100 micromolar (µM) isopropyl β-D-1-thiogalactopyranoside (IPTG) for 16:00:00 at 18 °C. 16h

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

Lysis buffer:

	A	B
	Tris-HCl pH 7.4	50 mM
	NaCl	300 mM
	Triton X-100	1%
	Glycerol	5%
	MgCl2	2 mM
	DTT	1 mM
	β-mercaptoethanol	2 mM
	cOmplete EDTA-free protease inhibitors (Roche)	
	CIP protease inhibitor (Sigma)	
	DNase (Sigma)	

5 Sonicate the cell lysates twice for 00:00:30 and clear by centrifugation at 18000 rpm, 4°C, 00:45:00 in a SORVAL RC6+ centrifuge with an F21S-8x50Y rotor (Thermo Scientific). 2h 45m 30s

- Collect the supernatant and incubate with pre-equilibrated Glutathione Sepharose 4B beads (GE Healthcare) for 02:00:00 at 4 °C with gentle shaking to bind FKBP8-GST.

6 Centrifuge the samples to pellet the beads and remove the unbound lysate. Then wash the beads twice with wash buffer, once with high salt wash buffer, and two more times with wash buffer.

Wash Buffer:

	A	B
	Tris-HCl pH 7.4	50 mM
	NaCl	300 mM
	DTT	1 mM

Salt wash Buffer:

	A	B
	Tris-HCl pH 7.4	50 mM
	NaCl	700 mM
	DTT	1 mM

7 Incubate the beads Overnight with 4 mL of [M] 50 millimolar (mM) reduced glutathione dissolved in wash buffer at 4 °C, to elute FKBP8-GST from the beads. 8h

Wash Buffer:

	A	B
	Tris-HCl pH 7.4	50 mM
	NaCl	300 mM
	DTT	1 mM

8 To collect the supernatant, collect the beads by centrifugation. Wash the beads twice with 4 mL of wash buffer, and collect the supernatant.

9 Pool the supernatant fractions, filter through a 0.45 µm syringe filter, concentrated with 30 kDa cut-off Amicon filter (Merck Millipore), and load onto a pre-equilibrated Superdex 200 Increase 10/300 GL column (Cytiva).

10 Elute the proteins with SEC buffer.

SEC Buffer:

	A	B
	Tris-HCl pH 7.4	25 mM
	NaCl	300 mM
	DTT	1 mM

11 Analyze the fractions by SDS-PAGE and Coomassie staining. Pool the fractions containing purified FKBP8-GST.

12 After concentrating the purified protein, aliquot the protein and snap-frozen in liquid nitrogen.

13 Store proteins at -80 °C.