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Purification of GST-LC3B

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Abstract

This protocol describes the purification of GST-LC3B.

Attachments

PDF

ppgyb32rp.pdf
29KB

Materials

Materials:

- pGEX-4T1 vector (LC3B-encoding vector is available from Addgene)
- Glutathione Sepharose 4B beads (GE Healthcare)
- Amicon filter (Merck Millipore)
- Superdex 75 16/600 column (Cytiva)

Lysis buffer:

	A	B
	HEPES pH 7.5	50 mM
	NaCl	300 mM
	MgCl2	2 mM
	βmercaptoethanol	2 mM
	cOmplete EDTA-free protease inhibitors (Roche)	
	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

	A	B
	NaCl	150 mM
	DTT	1 mM

Equipment

- Beckman Ti45 rotor

Purification of GST-LC3B

8h 30m 30s

1 To purify GST-LC3B, insert human LC3B cDNA in a pGEX-4T1 vector. Note, the last five amino acids of LC3B are deleted to mimic the cleavage by ATG4.

2 After the transformation of the pGEX-4T1 vector encoding GST-LC3B in E.coli Rosetta (DE3) pLysS cells, grow the cells in LB medium at 37 °C until an OD_{600} of 0.8-1 and induce the protein expression with [M] 1 millimolar (mM) IPTG for 04:00:00 at 37 °C. 4h

3 Collect cells by centrifugation and resuspend in lysis buffer.

Lysis buffer:

	A	B
	HEPES pH 7.5	50 mM
	NaCl	300 mM
	MgCl2	2 mM
	βmercaptoethanol	2 mM
	cOmplete EDTA-free protease inhibitors (Roche)	
	DNase (Sigma)	

4 Sonicate cell lysates. 1m

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

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

5 Clear lysates by centrifugation at 140000 x g, 4°C, 00:30:00 in a Beckman Ti45 rotor. 30m

6 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 GST-LC3B. 2h

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

8 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

9 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. 8h

10 Collect the supernatant, filter through a 0.45 µm syringe filter, concentrate using a 10 kDa cut-off Amicon filter (Merck Millipore), and load onto a pre-equilibrated Superdex 75 16/600 column (Cytiva).

11 Elute the proteins with SEC buffer.

SEC buffer

	A	B
	HEPES pH 7.4	25 mM

	A	B
	NaCl	150 mM
	DTT	1 mM

12 Analyze fractions by SDS-PAGE and Coomassie staining.

13 Pool fractions containing purified GST-LC3B protein.

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