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Purification of GST-tagged linear tetra-ubiquitin (4xUb)

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We use this protocol and it's working

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Abstract

This protocol describes purification of GST-tagged linear tetra-ubiquitin (4xUb).

Attachments

PDF

774-1960.pdf
59KB

Materials

Materials

- pGEX-4T1 vector (RRID:Addgene #199779)
- isopropyl β-D-1-thiogalactopyranoside (IPTG)
- Glutathione Sepharose 4B beads (GE Healthcare)
- 10 kDa cut-off Amicon filter (Merck Millipore)
- Superdex 200 Increase 10/300 GL column (Cytiva)
- SORVAL RC6+ centrifuge with an F21S8×50Y rotor (Thermo Scientific)

Lysis buffer

	A	B
	Tris-HCl pH 7.4	50 mM
	NaCl	300 mM
	MgCl2	2 mM
	DTT	1 mM
	cOmplete EDTA-free protease inhibitors (Roche)	
	DNase (Sigma)	

Wash buffer

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

High salt wash buffer

	A	B
	Tris-HCl pH 7.4	50 mM

	A	B
	NaCl	700 mM
	DTT	1 mM

SEC buffer

	A	B
	Tris-HCl pH 7.4	25 mM
	NaCl	150 mM
	DTT	1 mM

Purification of GST-tagged linear tetra-ubiquitin (4xUb)

18h 46m

1 Linear tetra-ubiquitin fused to GST (GST-4xUb) was cloned into a pGEX-4T1 vector and is available from Addgene (RRID:Addgene #199779).

2 After the transformation of the pGEX-4T1 vector encoding GST4xUb in *E. coli* Rosetta pLySS cells, grow cells in LB medium at 37 °C until an OD_{600} of 0.4 and then continued at 18 °C.

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

4 Collect cells by centrifugation and resuspend in lysis buffer.

5 Sonicate cell lysates.

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

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

6 Clear lysates by centrifugation at 18000 rpm, 4°C, 00:45:00 in a SORVAL RC6+ centrifuge with an F21S8×50Y rotor (Thermo Scientific). 45m

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

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

9 Wash the beads.

9.1 Wash the beads twice with wash buffer.

9.2 Wash the beads once with high salt wash buffer.

9.3 Wash the beads twice with wash buffer.

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

11 To collect the supernatant, collect the beads by centrifugation.

12 Wash the beads twice with 4 mL of wash buffer, and collect the supernatant.

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

14 Elute the proteins with SEC buffer.

15 Analyze the fractions by SDS-PAGE and Coomassie staining.

16 Pool the fractions containing purified GST-4xUb.

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