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Purification of NAP1 or GST-NAP1

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We use this protocol and it's working

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Abstract

This protocol describes purification of GST-NAP1 and unlabelled NAP1.

Attachments

840-2174.pdf
49KB

Materials

Materials

- pGEX-4T1 vector
- Glutathione Sepharose 4B beads (GE Healthcare)
- Superose 6 Increase 10/300 GL column (Cytiva)
- Amicon filter (Merck Millipore)

Lysis buffer

	A	B
	Tris-HCl pH 7.4	50 mM
	NaCl	300 mM
	DTT	1 mM
	$MgCl_2$	2 mM
	glycerol	5%
	β-mercaptoethanol	2 mM
	cOmplete EDTA-free protease inhibitors (Roche)	
	DNase (Sigma)	

Wash buffer

	A	B
	Tris-HCl pH 7.4	50 mM
	NaCl	300 mM
	glycerol	5%
	DTT	1 mM

High-salt wash buffer

	A	B
	Tris-HCl pH 7.4	50 mM
	NaCl	700 mM
	glycerol	5%

	A	B
	DTT	1 mM

SEC buffer

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

Equipment

SORVAL RC6+ centrifuge
F21S-8×50Y rotor (Thermo Scientific)

Purification of NAP1 or GST-NAP1

18h 46m

1 To purify NAP1 or GST-NAP1, synthesize or clone human NAP1 cDNA in a pGEX-4T1 vector with an N-terminal GST tag followed by a TEV cleavage site (RRID:Addgene_208870).

2 For expression of GST-TEV-NAP1 in *E. coli*, transform the pGEX-4T1 vector encoding GST-TEV-NAP1 into *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 16:00:00 at 18 °C. 16h

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

Lysis buffer

	A	B
	Tris-HCl pH 7.4	50 mM
	NaCl	300 mM
	DTT	1 mM
	$MgCl_2$	2 mM
	glycerol	5%
	β-mercaptoethanol	2 mM
	cOmplete EDTA-free protease inhibitors (Roche)	
	DNase (Sigma)	

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. (1/2) 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 GST-TEV-NAP1. 2h

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

9 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
	glycerol	5%
	DTT	1 mM

High-salt wash buffer

	A	B
	Tris-HCl pH 7.4	50 mM
	NaCl	700 mM
	glycerol	5%
	DTT	1 mM

10 Incubate beads Overnight at 4 °C with TEV protease (for unlabeled NAP1) or 4 mL of [M] 50 millimolar (mM) reduced glutathione dissolved in wash buffer (for GST-NAP1). 2h

11 After the proteins were released from the beads, filter the GST-NAP1 protein through a 0.45 µm syringe filter, concentrated using a 30 kDa cut-off Amicon filter (Merck

Millipore), or 10 kDa cut-off in case of unlabeled NAP1, and loaded onto a pre-equilibrated Superose 6 Increase 10/300 GL column (Cytiva).

12 Elute proteins with SEC buffer.

SEC buffer

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

13 Analyze fractions by SDS-PAGE and Coomassie staining.

14 Pool fractions containing purified NAP1 or GST-NAP1 protein.

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