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Purification of MBP-NAP1

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We use this protocol and it's working

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Abstract

This protocol describes purification of MBP-TEV-NAP1 protein.

Attachments

839-2173.pdf
48KB

Materials

Reagents

- NAP1 WT (RRID:Addgene_208871)
- NAP1 delta-NDP52 (S37K/A44E) (RRID:Addgene_208872)
- NAP1 delta-TBK1 (L226Q/L233Q) (RRID:Addgene_208873)
- pGEX-4T1 vector
- 2xTY medium
- D-maltose (Santa Cruz)
- IPTG
- Amylose beads (Biolabs)

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%
	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)
- Amicon filter (Merck Millipore)
- Superose 6 Increase 10/300 GL column (Cytiva)

Purification of MBP-NAP1

20h 46m

1 To purify MBP-NAP1, gene-synthesize human NAP1 cDNA (by Genscript) and subclone into a pGEX-4T1 vector with an N-terminal MBP-tag. Follow it by a TEV cleavage site before wild-type NAP1 (RRID:Addgene_208871), NAP1 delta-NDP52 (S37K/A44E) (RRID:Addgene_208872), or NAP1 delta-TBK1 (L226Q/L233Q) (RRID:Addgene_208873).

2 For expression of MBP-TEV-NAP1 in *E. coli*, transfer the pGEX-4T1 vector encoding MBP-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 them 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 and then clear by centrifugation.

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

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

5.3 Then, centrifugation at 18000 rpm, 4°C, 00:45:00 in a SORVAL RC6+ centrifuge with an F21S-8×50Y rotor (Thermo Scientific). 45m

6 Collect and incubate the supernatant with pre-equilibrated Amylose beads (Biolabs) for 02:00:00 at 4 °C with gentle shaking to bind MBP-TEV-NAP1. 2h

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

8 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

9 Incubate the beads Overnight at 4 °C with [M] 250 millimolar (mM) D-maltose (Santa Cruz) dissolved in wash buffer. 2h

10 After the proteins are released from the beads, filter the MBP-TEV-NAP1 protein through a 0.45 µm syringe filter, concentrate using a 30 kDa cut-off Amicon filter (Merck Millipore), and load onto a pre-equilibrated Superose 6 Increase 10/300 GL column (Cytiva).

11 Elute the proteins with SEC buffer.

SEC buffer

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

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

13 Pool the fractions containing purified MBP-TEV-NAP1 protein.

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