

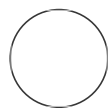


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## Purification of MBP-NAP1

Elias Adriaenssens<sup>1</sup>

<sup>1</sup>Sascha Martens lab, University of Vienna, Max Perutz Labs - Vienna



Elias Adriaenssens

Sascha Martens lab, University of Vienna, Max Perutz Labs - ...

### ABSTRACT

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

### ATTACHMENTS

[839-2173.pdf](#)

### 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 <sub>2</sub>	2 mM
glycerol	5%
β-mercaptoethanol	2 mM
cOmplete EDTA-free protease inhibitors (Roche)	
DNase (Sigma)	

OPEN ACCESS



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**Protocol status:** Working  
 We use this protocol and it's working

**Created:** Sep 14, 2023

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**Keywords:** ASAPCRN

### 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-8x50Y 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<sub>600</sub> of 0.4 and then continue at  18 °C .

3 Once the cells reached an OD<sub>600</sub> of 0.8, induce protein expression with  50 micromolar (μM)  16h  
IPTG for  16:00:00 at  18 °C .



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 <sub>2</sub>	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 45m



6 Collect and incubate the supernatant with pre-equilibrated Amylose beads (Biolabs) for 2h



 02:00:00 at  4 °C with gentle shaking to bind MBP-TEV-NAP1.

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

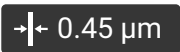
A	B
Tris-HCl pH 7.4	50 mM
NaCl	300 mM
glycerol	5%
DTT	1 mM

#### 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  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.

