

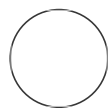


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## Purification of NAP1 or GST-NAP1

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### ABSTRACT

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

### ATTACHMENTS

[840-2174.pdf](#)

### 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 <sub>2</sub>	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

OPEN ACCESS



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[dx.doi.org/10.17504/protocols.io.kqdg3xk41g25/v1](https://dx.doi.org/10.17504/protocols.io.kqdg3xk41g25/v1)

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

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**PROTOCOL integer ID:**  
88168

**Keywords:** ASAPCRN

A	B
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)

## 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<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 [IM] 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

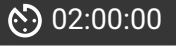

A	B
Tris-HCl pH 7.4	50 mM
NaCl	300 mM
DTT	1 mM
MgCl <sub>2</sub>	2 mM
glycerol	5%
β-mercaptoethanol	2 mM
cOmplete EDTA-free protease inhibitors (Roche)	
DNase (Sigma)	

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-8x50Y 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.



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





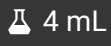
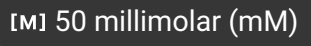
- 9 Wash the beads twice with wash buffer, once with high salt wash buffer, and two more times with wash buffer.

#### Wash buffer

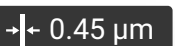
A	B
Tris-HCl pH 7.4	50 mM
NaCl	300 mM
glycerol	5%
DTT	1 mM

#### 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  50 millimolar (mM) reduced glutathione dissolved in wash buffer (for GST-NAP1).



- 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 .

