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## NAP1-mCherry

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We use this protocol and it's working

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## Abstract

This protocol describes how to express and purify human NAP1 tagged C-terminally with mCherry.

## Attachments



Purification of NAP1...

990KB



## Materials

### Expression:

pCAGG\_GST-TEV-NAP1-mCherry (Addgene ID: 198036)

FreeStyle™ 293-F Cells

FreeStyle™ 293 Expression Medium (Thermo, 12338-026)

Opti-MEMR I Reduced Serum Medium (Thermo, 31985-062)

Polyethylenimine (PEI 25K, Polysciences CatNo 23966-1)

EXCELLR 293 Serum-Free Medium (SigmaAldrich, 14571C-1000ML)

PBS

### Lysis Buffer:

50 mM Tris-HCl pH 8.0

300 mM NaCl

2 mM MgCl<sub>2</sub>

5% Glycerol

2 mM b-Met

Complete inhibitor EDTA free Roche

50ul of Protease inhibitors Sf9 cells

Benzonase (1ul)

### Wash I Buffer:

50 mM Tris-HCl pH 8.0

300 mM NaCl

5% Glycerol

1 mM DTT

### Wash II Buffer:

50 mM Tris-HCl pH 8.0

700 mM NaCl

5% Glycerol

1 mM DTT

### SEC Buffer:

20 mM Tris-HCl pH 7.4

300 mM NaCl

1 mM DTT

### Columns/Resin:

Glutathione Sepharose 4B (Cytiva)

Superose 6 Increase 10/300 column (Cytiva)

## Expression

- 1 To generate GST-TEV-NAP1-mCh the gene coding for the protein sequence of human NAP1 was subcloned together with N-terminal GST-TEV and C-terminal mCherry tags into a pCAGG. The construct is available at AddGene with the ID: 198036.
- 2 The protein was expressed in FreeStyle™ 293-F Cells by transient transfection. The cells grown at 37°C in FreeStyle™ 293 Expression Medium (Thermo, 12338-026) were seeded to density of  $0.7 \times 10^6$  cells per ml the day before transfection. On the day of transfection, 400 ml culture at density of  $1 \times 10^6$  cells per ml was transfected by addition of vortexed transfection mixture. The mixture consisted of two components: 400 ug of the MAXI-prep DNA that was pre-diluted in 13 ml of FreeStyle™ 293 Expression Medium (Thermo, 12338-026) and 800 ug Polyethylenimine (PEI 25K, Polysciences CatNo 23966-1) likewise pre-diluted in 13 ml of Opti-MEM media. 24 hours post transfection, the culture was fed by addition of 100 ml of EXCELLR 293 Serum-Free Medium (SigmaAldrich, 14571C-1000ML). 24H post transfection, the cells were harvested by centrifugation at 270 g for 20 minutes, washed by 1xPBS and flash-frozen in liquid nitrogen prior to storage at -80 °C.

## Purification

- 3 Thaw a cell pellet corresponding to 1L culture by re-suspending it in 25 ml lysis buffer (50 mM Tris-HCl pH 8.0, 300mM NaCl, 2 mM MgCl<sub>2</sub>, 5% glycerol, 2 mM β-Met, 1 μl Benzonase (Sigma), CIP protease inhibitor (Sigma), cOmplete EDTA-free protease inhibitor cocktail (Roche)) and rolling or stirring in the cold room.
- 4 Additionally disrupt the cells with a Dounce homogenizer and two rounds of 30s sonication on ice.
- 5 Clear the lysate by centrifugation (10 000 rpm for 45 min at 4°C in a Fiberlite F21-8×50y (Thermo Scientific)).
- 6 Incubate the cleared supernatant with 2 ml of Glutathione Sepharose 4B beads slurry (Cytiva) for 2h at 4°C rolling gently. The GSH slurry should be washed with water and then with Wash I Buffer beforehand (50 mM Tris-HCl pH 8.0, 300 mM NaCl, 5% glycerol, 1 mM DTT).
- 7 After 2h of incubation with the cleared lysate wash the beads two times with Wash I Buffer (50 mM Tris-HCl pH 8.0, 300 mM NaCl, 5% glycerol, 1 mM DTT), once with Wash II buffer (50 mM Tris-HCl pH 8.0, 700 mM NaCl, 5% glycerol, 1 mM DTT) and again twice with Wash I Buffer.



- 8 Incubate the beads overnight with TEV protease at 4°C (20 ul of 10 mg/ml home-made TEV).
- 9 The next day spin down the beads (4000 rpm, 3 min, 4°C) and collect the supernatant containing cleaved NAP-mCh.
- 10 Filter the supernatant through a 0.45 µm syringe filter to remove any residual beads.
- 11 Concentrate the protein down to 0.5 ml using a 30kDa cut-off Amicon filter and apply onto a Superose 6 Increase column (10/300, Cytiva) pre-equilibrated with a SEC buffer containing 20 mM Tris-HCl, pH 7.4, 300 mM NaCl, and 1 mM DTT. Pool fractions containing pure proteins (see attached pdf), concentrate, snap freeze in liquid nitrogen, and store at -80°C.