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Expression and purification of mCherry-NDP52

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes how to express in *E.Coli* human NDP52 N-terminally tagged with mCherry and purify it through His affinity purification followed by Size Exclusion Chromatography.

Materials

Protein information:

Molecular weight: 80384.47 Da

Ext. coefficient: $77240 \text{ M}^{-1}\text{cm}^{-1}$

Abs 0.1% (=1 g/l) 0.961, assuming all Cys residues are reduced

Expression:

pETDuet-1_6xHis-TEV-mCh-NDP52 (Addgene ID: 187829)

E. coli Rosetta pLysS cells

LB medium with antibiotics: 50 µl/ml ampicillin and 34 µl/ml chloramphenicol

IPTG (Isopropyl-β-D-thiogalactopyranosid)

Lysis Buffer:

50 mM HEPES pH 7.5

300 mM NaCl

2 mM MgCl₂

2 mM b-Met

Complete inhibitor EDTA free Roche

DNase

Buffer A:

50mM HEPES pH 7.5

300 mM NaCl

10 mM Imidazole

2 mM Beta-Mercaptoethanol

Buffer B:

50mM HEPES pH 7.5

300 mM NaCl

300 mM Imidazole

2 mM Beta-Mercaptoethanol

SEC Buffer:

25 mM HEPES pH 7.5

150 mM NaCl

1 mM DTT

Columns/Resin:

5-ml His-Trap column (Cytiva)

Superdex 200 increase 16/600 column (Cytiva)



Expression

- 1 pETDuet-1_6xHis-TEV-mCh-NDP52 (Addgene ID: 187829) was transformed into *E. coli* Rosetta pLySS cells.
- 2 To express the protein grow *E. coli* Rosetta pLySS cells in 4 L of LB medium (w Amp/Cam) at 37°C until an OD_{600 nm} of 0.4. Next, bring the temperature down to 18°C and grow further to an OD_{600 nm} of 0.8. Induce protein expression with 0.5 mM IPTG and grow for further 16 h at 18°C.
- 3 Pellet the cells at 4000 rpm  4 °C  00:15:00 . Re-suspended the cell pellet in a buffer containing 50mM HEPES pH7.5, 300 mM NaCl, 1 mM MgCl₂, 10 mM Imidazole, 2 mM Beta-Mercaptoethanol, cOmplete protease inhibitors (Roche), and DNase (Sigma). Snap freeze in liquid nitrogen and store in  -80 °C until the day of purification.

15m

Purification

- 4 Open the cells by thawing in RT water bath and sonicating 3 × 30 seconds at 50% power using a Bandelin sonicator.
- 5 Clear the lysate by ultracentrifugation (40,000 rpm for 45 min at 4°C in a Ti45 rotor, Beckman).
- 6 Filter the supernatant with a 0.45 µm syringe filter on ice.
- 7 Apply to a 5-ml His-Trap column (Cytiva) and elute with a stepwise imidazole gradient (50, 75, 100, 150, 200, and 300 mM). Fractions at 75–100 mM imidazole should contain His-TEV-mCh-NDP52. Pool those fractions and subject to TEV protease cleavage over night at 4°C by very gentle rolling.
- 8 After TEV cleavage, concentrate the protein using a 50kDa cut-off Amicon filter to 0.5 ml and inject onto a Superdex 200 increase 16/600 column (Cytiva) pre-equilibrated with a buffer containing 25mM HEPES pH7.5, 150mM NaCl, 1mM DTT at 4°C.
- 9 Pool fractions containing pure protein, concentrate using a 50kDa cut-off Amicon filter, snap freeze in liquid nitrogen, and store at -80°C.