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Expression and purification of mCherry-NDP52

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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.

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KEYWORDS

mCherry-NDP52, mCh-NDP52, NDP52, E. Coli, Expression , Purification, His Affinity , Size Exclusion Chromatography

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MATERIALS TEXT

Protein information:
Molecular weight: 80384.47 Da
Ext. coefficient: 77240 $M^{-1}*cm^{-1}$
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 15m pH7.5, 300 mM NaCl, 1 mM $MgCl_2$, 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.

Purification

4 Open the cells by thawing in RT water bath and sonicating 3 x 30 seconds at 50% power using a Bandelin sonicator.

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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-equillibrated 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.

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