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NDP52 and OPTN S177D S473D: expression and purification

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ABSTRACT

This protocol describes how to express and purify human NDP52 and OPTN S177D S473D. The same procedure can be applied to purify wild type OPTN.

ATTACHMENTS

protocols io NDP52 and OPTN.pdf

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PROTOCOL CITATION

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KEYWORDS

NDP52 purification, OPTN purification, optineurin purification

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MATERIALS TEXT

Expression:
pGST2-GST-TEV-NDP52 (Addgene ID: 187828)
pGST2-GST-TEV-OPTN S177D S473D (Addgene ID: 187827)
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

Wash Buffer I (low salt):
50 mM HEPES pH 7.5
300 mM NaCl
1 mM DTT

Wash Buffer II (high salt):
50 mM HEPES pH 7.5
700 mM NaCl
1 mM DTT

SEC Buffer:
25 mM HEPES pH 7.5
150 mM NaCl
1 mM DTT

Columns/Resin:
Glutathione Sepharose 4B (Cytiva)
Superose 6 increase 10/300 column (Cytiva)

Constructs information

1 Human NDP52 and human OPTN S177D S473D genes were cloned into bacterial expression vector. Addgene IDs: 187828 and 187827, respectively.

Expression 30m

2 The proteins were expressed in *E. coli* Rosetta pLySS cells. Grow the cells in 4 L of LB medium at 37°C until an $OD_{600\ nm}$ of 0.4. Next, bring the temperature down to 18°C and grow further to

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an $OD_{600\ nm}$ of 0.8. Induce protein expression with 100 µM 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 lysis buffer containing 50 mM HEPES, pH 7.5, 300 mM NaCl, 2 mM MgCl2, 2 mM β-mercaptoethanol, cOmplete protease inhibitors (Roche), DNase and flash freeze in liquid nitrogen. Store in **-80 °C** until the day of purification.

Purification 15m

4 Open the cells by thawing and sonicating 2 x 30 seconds.

5 Clear the lysate by centrifugation (25 000 rpm for 30 min at 4°C in a Ti45 rotor, Beckman).

6 Incubate the cleared supernatant with 5 ml of Glutathione Sepharose 4B beads slurry (Cytiva) for 1h at 4°C rolling slowly. The beads slurry should be washed with water and then with 50 mM HEPES, pH 7.5, 300 mM NaCl, and 1 mM DTT beforehand.

7 After 1h of incubation with the cleared lysate wash the beads four times with 40 ml low salt buffer (50 mM HEPES, pH 7.5, 300 mM NaCl, and 1 mM DTT) buffer, followed by one wash with high salt buffer (50 mM HEPES, pH 7.5, 700 mM NaCl, and 1 mM DTT) and again two washes with low salt buffer.

8 Finally, incubate **Overnight** with TEV protease at 4°C. 20 ul of 10 mg/ml home-madeTEV protease.

9 The next day spin down the beads (4000 rpm, 3 min, 4°C) and collect the supernatant containing cleaved protein.

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 30kDa cut-off Amicon filter and apply onto a Superose 6 increase column (10/300 Cytiva) pre-equillibrated with a buffer containing 25 mM HEPES, pH 7.5, 150 mM NaCl, and 1 mM DTT.

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12 Pool fractions containing pure proteins (see attached pdf), concentrate, snap freeze in liquid nitrogen, and store at −80°C.

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