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We use this protocol and it's working

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ABSTRACT

This protocol describes purification of NDP52 (untagged).

ATTACHMENTS

775-1961.pdf

MATERIALS

Materials

- Human NDP52 cDNA in pGST2 vector with an N-terminal GST tag (RRID:Addgene #187828)
- IPTG
- SORVAL RC6+ centrifuge with an F21S8x50Y rotor (Thermo Scientific)
- Glutathione Sepharose 4B beads (GE Healthcare)
- 30 kDa cut-off Amicon filter (Merck Millipore)
- Superdex 200 Increase 10/300 GL column (Cytiva)

Lysis buffer

	A	B
	Tris-HCl pH 7.4	50 mM
	NaCl	300 mM
	DTT	1 mM
	MgCl2	2 mM
	β-mercaptoethanol	2 mM
	cOmplete EDTA-free protease inhibitors (Roche)	
	DNase (Sigma)	

Wash buffer

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

High salt wash buffer

	A	B
	Tris-HCl pH 7.4	50 mM

	A	B
	NaCl	700 mM
	DTT	1 mM

SEC buffer

	A	B
	Tris-HCl pH 7.4	25 mM
	NaCl	150 mM
	DTT	1 mM

Purification of NDP52 (untagged)

16h

1 The human NDP52 cDNA into a pGST2 vector with an N-terminal GST tag followed by a TEV cleavage site is available from Addgene (RRID: Addgene #187828).

2 After the transformation of the pGST2 vector encoding GST-TEV-NDP52 in *E. coli* Rosetta pLySS cells, grow cells in 2xTY medium at 37 °C until an OD_{600} of 0.4 and then continued at 18 °C.

3 Once the cells reached an OD_{600} of 0.8, induce protein expression with [M] 50 micromolar (µM) IPTG for 16:00:00 at 18 °C. 16h

4 Collect cells by centrifugation and resuspend in lysis buffer.

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 lysates by centrifugation at 18000 rpm, 4°C, 00:45:00 in a SORVAL RC6+ centrifuge with an F21S8x50Y rotor (Thermo Scientific). 45m

7 Collect the supernatant and incubate with preequilibrated Glutathione Sepharose 4B beads (GE Healthcare) for 02:00:00 at 4 °C with gentle shaking to bind GST-NDP52. 2h

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

9 Wash the beads.

9.1 Wash the beads twice with wash buffer.

9.2 Wash the beads with high salt wash buffer.

9.3 Again, wash the beads twice with wash buffer.

10 Incubate beads Overnight with TEV protease at 4 °C.

11 After the GST tag was cleaved off, filter the protein through a 0.45 µm syringe filter, concentrated using a 30 kDa cut-off Amicon filter (Merck Millipore), and load onto a pre-equilibrated Superdex 200 Increase 10/300 GL column (Cytiva).

12 Elute proteins with SEC buffer.

13 Analyze fractions by SDS-PAGE and Coomassie staining.

14 Pool fractions containing purified NDP52.

15 After concentrating the purified protein, aliquot the protein and snap-freeze in liquid nitrogen. Store the proteins at -80 °C.