Purification of NDP52 (untagged)

Human NDP52 cDNA was cloned into a pGST2 vector with an N-terminal GST tag followed by a TEV cleavage site (RRID:Addgene #187828). After the transformation of the pGST2 vector encoding GST-TEV-NDP52 in *E. coli* Rosetta pLySS cells, cells were grown in 2xTY medium at 37°C until an OD_{600} of 0.4 and then continued at 18°C. Once the cells reached an OD_{600} of 0.8, protein expression was induced with 50 µM IPTG for 16 h at 18°C. Cells were collected by centrifugation and resuspended in lysis buffer (50 mM Tris-HCl pH 7.4, 300 mM NaCl, 1 mM DTT, 2 mM $MgCl_2$, 2 mM β-mercaptoethanol, cOmplete EDTA-free protease inhibitors (Roche), and DNase (Sigma)). Cell lysates were sonicated twice for 30 s. Lysates were cleared by centrifugation at 18,000 rpm for 45 min at 4°C in a SORVAL RC6+ centrifuge with an F21S-8x50Y rotor (Thermo Scientific). The supernatant was collected and incubated with pre-equilibrated Glutathione Sepharose 4B beads (GE Healthcare) for 2 h at 4°C with gentle shaking to bind GST-NDP52. Samples were centrifuged to pellet the beads and remove the unbound lysate. Beads were then washed twice with wash buffer (50 mM Tris-HCl pH 7.4, 300 mM NaCl, 1 mM DTT), once with high salt wash buffer (50 mM Tris-HCl pH 7.4, 700 mM NaCl, 1 mM DTT), and two more times with wash buffer (50 mM Tris-HCl pH 7.4, 300 mM NaCl, 1 mM DTT). Beads were incubated overnight with TEV protease at 4°C. After the GST tag was cleaved off, the protein was filtered through a 0.45 µm syringe filter, concentrated using a 30 kDa cut-off Amicon filter (Merck Millipore), and loaded onto a pre-equilibrated Superdex 200 Increase 10/300 GL column (Cytiva). Proteins were eluted with SEC buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM DTT). Fractions were analyzed by SDS-PAGE and Coomassie staining. Fractions containing purified NDP52 were pooled. After concentrating the purified protein, the protein was aliquoted and snap-frozen in liquid nitrogen. Proteins were stored at -80°C.