

NET1

PDB:3EO2

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:NP_001040625.1

Entry Clone Source:MGC AT79-C6

SGC Clone Accession:HPC083-D09

Tag:N-terminal tag: mhhhhhhsgrenlyfq*g

Host:BL21(DE3)-V2R-pRARE2

Construct

Prelude:

Sequence:

mhhhhhhsgrenlyfqgDITMKESLTREIRRQEAIYEMSRGEQDLIEDLKLARKAYHDPMLKLSIMSEEELTHIFGDLD SYIPLH
EDLLTRIGEATKPDGTVEQIGHILVWLPRLNAYRGYCSNQLAAKALLDQKKQDPRVQDFLQRCLES PFSRKLDLWSFLDIPRSRLV
KYPLLKEILKHTPKEHPDVQLLEDAILIIQGVLS DINLKKGESECQYYIDKLEYLD

Vector:pET28-mhl (GI:134105571)

Growth

Medium:Terrific Broth

Antibiotics:

Procedure:LEX Bubbling. The target protein was expressed in *E. coli* by inoculating 60 mL of overnight culture grown in Luria-Bertani medium into a 1.8 L of Terrific Broth medium in the presence of 50 µg/mL kanamycin and 25 µg/mL Chloramphenicol at 37 degC. When OD₆₀₀ reached ~3.0, the temperature of the medium was lowered to 15 degC and the culture was induced with 0.5 mM IPTG. The cells were allowed to grow overnight before they were harvested and flash frozen in liquid nitrogen and stored at -80 degC.

Purification

Procedure

The lysate was centrifuged at 15,000 rpm for 45 minutes and the supernatants were mixed with 3 mL 50% Ni-NTA beads, and incubated at 4 degC for 1 hours. The supernatant was then passed through a gravity column (Poly-Prep, Bio-Rad, Catalog #731-1550) and the beads were washed using 15 mL washing buffers(contains 5mM, 30mM or 75 mM Imidazole separately). The protein bound to beads were eluted using 15 mL elution buffer once. The flow-through fractions washed using buffer containing 30mM, 75mM, 300mM Imidazole were collected and loaded onto

Supderdex-75 gel filtration column. Eluted fractions were pooled and concentrated using amicon centrifugal filter (m.w. cut-off 10,000). The purity of the proteins was higher than 95% judged by SDS-PAGE.

Extraction

Procedure

Frozen cells from 1.8L TB culture were thawed and resuspended in 150 mL extraction buffer with freshly added 0.5% CHAPS and 2mM BME, and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 3 microL benzonase (Sigma Catalog # E1014, 250U/microL), and lysed using microfluidizer.

Concentration:32.6mg/mL

Ligand

MassSpec:

Crystallization:Initial crystallization screen was setup using sitting drops with Red Wings and SGC-I screens and also with in situ proteolytic treatment. Crystals were seen at conditions SF02 and SB08 conditions. The optimized crystal used for data collection was grown in 1.0 M Sodium Citrate, 0.1 M Cacodylate buffer pH 4.8, 5% ethylene glycol and at the presence of 1:100(w/w) subtilisin in hanging drop setup. The crystals grow to a mountable size within two days and shows diamond. N-paratone was used as cryoprotectant.

NMR Spectroscopy:

Data Collection:

Data Processing: