RHPN2

PDB:2VSV

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:IMAGE:4830913
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal His-tag with TEV protease cleavage site
Host:BL21(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsMPRSIRFTAEEGDLGFTLRGNAPVQVHFLDPYCSASVAGAREGDYIVSIQLVDCKWLTLSEVMKL
LKSFGEDEIEMKVVSLLDEVTF

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Growth medium, induction protocol:10µl of a glycerol stock was inoculated into 5ml of TB medium (supplemented with 50µg/ml Kanamycin) and cultured at 37°C o/n in a shaking incubator (275 rpm). Next day 0.75 ml of o/n culture was used to inoculate 1 litre of TB medium (3x) and grown at 37°C with vigorous shaking (160 rpm) until the culture reaches an OD600 of 1.4. Temperature was reduced to 18°C, and cells were induced with IPTG at a concentration of 0.2 mM, and further cultivated for 16 hrs. Cells were harvested by centrifugation at 6500 rpm for 10 min, and the cell pellet was stored at -20°C until further use.

Purification

Procedure
Column 1: Ni-Sepharose 6 Fast FlowBuffers: Lysis buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 5 mM Imidazole; Wash buffer: 50 mM Tris-HCl pH 7.5, 500 mM NaCl, 5% Glycerol, 30 mM Imidazole; Elution buffer:50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 250 mM Imidazole. Note: All the buffers contain 0.5mM TCEP.Procedure: The column was packed with 2 ml of Ni-Sepharose 6 Fast Flow slurry and equilibrated with 15 ml of binding buffer. The supernatant was loaded onto the column and the column was washed with 20 ml of

binding buffer and then 20 ml of washing buffer. The protein was eluted with 10 ml of elution buffer.Column 2: SuperDex S-75 (GE/Amersham)Buffer: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP.Procedure: The eluted protein from the Ni-affinity column was loaded on the gel filtration column in GF buffer at 1.0 ml/min on an AKTA Purifier system. Eluted proteins were collected in 1 ml fractions.

Extraction

Procedure
Extraction buffer, extraction method. Lysis buffer: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 5 mM Imidazole, Complete® protease inhibitors (Roche, 1 tbl/50 ml). Frozen cell pellets were thawed and resuspended in a total volume of 30-40 ml of lysis buffer, and disrupted by using Avestin C-5 microfluidizer, and a supernatant containing the target protein was obtained by centrifugation at 21,000 (rpm) for 45 minutes.
Concentration: Concentration: The RHPN2A protein (in buffer; 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP) was concentrated to 33 mg/ml using Vivaspin 5K concentrators and stored at -80°C.
Ligand
MassSpec: Mass spec characterization: Corresponds to theoretical mass, as determined by ESI-TOF MS.
Crystallization: Crystallization: RHPN2A was crystallized at 20°C using the sitting-drop vapour diffusion method. Diffraction quality crystals were obtained by mixing 100nl of concentrated protein solution (33mg/ml) with 50nl reservoir solution (0.2M sodium acetate, 20% PEG3350, 10% ethylene glycol).
NMR Spectroscopy:
Data Collection: Data Collection: Crystals were flash frozen in liquid nitrogen in reservoir solution supplemented with ethylene glycol (final concentration 25% v/v). Diffraction data were collected to 1.8 Å on beam-line X10SA at the Swiss light source (SLS); Resolution: 1.8 Å.
Data Processing: