PTPRG

PDB:2H4V

Revision

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Entry Clone Accession:gi|18860898
Entry Clone Source: Purely Proteins Ltd.
SGC Clone Accession:
Tag:mhhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag.
Host:BL21 (DE3)R3 (Phage resistant strain)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmKQFVKHI GELYSNNQHGFSEDFEEVQRCTADMNITAE HSNHPENKHKNRYINILAYDHSRVK
LRPLP GKDSKHSDYINANYVDGYNKAKAYIATQGP LKSTFEDFWRMIWEQNTGIIVMITNLVEKG RRKCDQYWPTENSEEYGNI
IVTLKSTKIHA CYTVRRFSIRNTKVKKGQKGNPKGRQNERV VIQYHYTQWPDMGVPEYALPVLTFVRRSSA ARMPETGPVLVHC
SAGVGRTGTYIVIDSML QQIKDKSTVNVLGFLKHIRTQRNYLVQTEE QYIFIHDALLEAILGKETEV

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:

Purification

Procedure
Column 1 : Ion exchange - Nucleic acid removal. DEAE cellulose (DE52, Whatman), 10 g of resin in 2.5 x 20 cm column. The resin was hydrated in 2.5M NaCl, then washed with 20 ml binding buffer prior to loading the sample.
Supernatant was applied by gravity flow, followed by a wash with 100 ml binding buffer. The column flow-through was collected.

Column 2: Ni-affinity. Ni-NTA (Qiagen), 5 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer.
The flowthrough from column 1 was loaded by gravity flow on the Ni-NTA column. The column was then washed with 100 ml wash buffer at gravity flow. The protein was eluted by gravity flow

by applying 5-ml portions of elution buffer with increasing concentration of imidazole (50 mM, 100 mM, 150 and 250 mM); fractions were collected until essentially all protein was eluted.

Enzymatic treatment : (His tag cleavage using TEV) Samples containing PTPRG were pooled and TEV protease added for overnight incubation at 4°C. Cleaved products and TEV protease were removed by binding to Ni-NTA agarose after buffer exchange to 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP using a 10 kDa cut-off concentrator.

Column 3: Size Exclusion Chromatography. Superdex S200 16/60 HiLoad
TEV-cleaved PTPRG, after re-binding to NiNTA, was directly applied to a S200 16/60 HiLoad gel filtration column equilibrated in 50 mM HEPES pH 7.5, 300 mM NaCl, 10 mM DTT using either an ÄKTAprime or ÄKTAxpress system

Extraction

Procedure
Frozen pellets were thawed and cells lysed using a high pressure cell disrupter. The lysate was centrifuged at 17,000 rpm for 30 minutes and the supernatant collected for purification.
Concentration: Protein was concentrated to 11.7 mg/ml using an Amicon 10 kDa cut-off concentrator
Ligand
MassSpec:LC- ESI -MS TOF confirmed the correct mass expected for this construct.
Crystallization: Crystals were grown at 20°C in 300nl sitting drops mixing 200 nl of protein with 100 nl of a solution containing 0.20M NH4(acetate); tris-sodium citrate dihydrate, pH 5.6; 30% PEG-4000.
NMR Spectroscopy:
Data Collection: Resolution: 2.4 Å; Crystals were cryo-protected using the well solution and 20% ethylene glycol and flash frozen in liquid nitrogen. X-ray source: Diffraction data were collected using a copper Ka radiation generated by a Rigaku FRE X-ray generator and a HTC detector.
Data Processing: