PTPRN2

PDB:2QEP

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|11386149
Entry Clone Source:MGC
SGC Clone Accession:
Tag:NON-cleavable C-terminal His 6 tag
Host: Phage-resistant derivative of BL21(DE3)

Construct

Prelude:
Sequence:

MSEEPVQSNMDISTGHMILSYMEDHLKNK NRLEKEWEALCAYQAEPNSSFVAQREENV PKNRSLAVLTYDHSRVLLKAENSHSHS
DY INASPIMDHDPRNPAYIATQGPLPATVAD FWQMVWESGCVVIVMLTPLAENGVRQCYH YWPDEGSNLYHIYEVNLVSEHIWC
EDFLV RSFYLKNLQTNETRTVTQFHFLSWYDRGV PSSSRSLLDFRRKVNKCYRGRSCPIIVHC SDGAGRSGTYVLIDMVLNKMA
KGAKEIDI AATLEHLRDQRPGMVQTKEQFEFALTAVA EEVNAILAhhhhhh

Vector:pNIC-CH.

Growth

Medium:LB
Antibiotics:
Procedure:1ml from a 10 ml overnight culture containing 50 µg/ml kanamycin was used to inoculate 1 litre of LB containing 50 µg/ml kanamycin. Cultures were grown at 37°C until the OD600 reached ~0.3 then the temperature was adjusted to 18°C. Expression was induced for 4 hours using 1 mM IPTG at an OD600 of 0.8. The cells were collected by centrifugation and the pellet resuspended in binding buffer and frozen.

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, washed with 20 ml binding buffer prior to loading the sample.
Column 2: Ni-affinity. Ni-NTA (Qiagen), 5 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer.
Column 3: Size Exclusion Chromatography. Superdex S200 16/60 HiLoad

Supernatant was applied by gravity flow with column 1, followed by a wash with 100 ml binding buffer. The column flow-through was collected.
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 mM and 250 mM); fractions were collected until essentially all protein was eluted. Eluted protein , was directly applied to a S200 16/60 HiLoad gel filtration column equilibrated in 50 mM Tris-HCl pH 7.5, 250 mM NaCl, 10 mM DTT using an AKTAxpress 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 40 mg/ml using an Amicon 10 kDa cut-off concentrator.
Ligand
MassSpec:LC- ESI -MS TOF indicated a loss of 130 a.m.u. corresponding to the proteolytic cleavage of the initial methionine. Expected mass : 34931; Observed mass: 34799
Crystallization: Crystals were grown at 20°C in 200 nl sitting drops mixing 100 nl of 40 mg/ml protein with 100 nl of a solution containing 0.2 M Na/KPO4; 0.1 M BTProp pH 6.5; 20.0% PEG 3350; 10.0% EtGly. The crystals were cryo-protected using 25% ethylene glycol which was added to the drop 30 seconds prior to mounting and flash freezing in liquid nitrogen.
NMR Spectroscopy:
Data Collection:Resolution: 2.5 Å. **X-ray source**: Diffraction data were collected at the SLS beamline X10SA at a single wavelength (0.9999 Å).
Data Processing: