PTPRTA: Human Protein Tyrosine Phosphatase Receptor T

PDB:2OOQ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi 5031765
Entry Clone Source:synthetic
SGC Clone Accession:
Tag:N terminus: mgsshhhhhhssgrenlyfqgh. C terminus: gs
Host:BL21(DE3)

Construct

Prelude:
Sequence:

Vector:p11

Growth

Medium:
Antibiotics:
Procedure: The 11β-HSD1 construct was expressed in the E. coli expression strain BL21(DE3), in TB medium with a plasmid encoding the chaperone GroEL/ES, under control of the arabinose promoter (Takara , Japan). High-level soluble protein production was achieved by IPTG induction (3 mM) at 18oC for 12 hrs in the presence of 1% sucrose, 1% glucose, 0.05% arabinose and 100 µM carbenoxolone (CBX, 3ß-hydroxy-11-oxoolean-12-en-30-oic acid, 3-hemisuccinate, Sigma).

Purification

Procedure
Column 1: HiTrap chelating 5ml_ Ni_Column
Procedure: Add supernatant to column at 3 ml/min, filtrate if necessary prior to application. Wash with buffer A to baseline. Wash with 9 % B. Wash with 16 % B. Go back to buffer A to baseline. Incubate column in buffer C at room temp over night. Wash with 9 % E. Wash with 16 % E to baseline. Elute with 60 % E

Column 2: Hi Load 16/60 Superdex 200

Extraction

Procedure
Collected/resuspended cells (50 mM Tris/Cl, 300 mM NaCl, 5 mM Imidazole, 2 mM TCEP, 0.5 uM CBX, 5% (w/v) glycerol, pH8.0) were disrupted in a high-pressure homogenizer, the supernatant after centrifugation at 15000 g for 40 min of the cell lysate was subjected to immobilized metal affinity chromatography, followed by incubation overnight in 0.05% Triton X-100 (Anapoe X-100; Anatrace, Maumee, OH), 20 µM CBX, 50 mM Tris/Cl, pH 8.0, 300 mM NaCl, 2 mM TCEP, 0.5 mM MgCl2, 2 mM ATP and a final gel filtration chromatographic step on a Superdex 200 HiLoad 16/60 column (GE Healthcare, Uppsala, Sweden). The protein was concentrated in 20 mM Tris-HCl, pH 8.0, containing 2mM TCEP, 5% (w/v) glycerol, 0.05 % (w/v) Triton X-100, and 2 µM of the inhibitor to around 12 mg/ml using a 30000 kDa MW cutoff Amicon Ultra concentration device (Millipore, Bedford, MA).
Concentration:12 mg/ml
Ligand
MassSpec:observed mass 31064 Da, theoretical mass 31063.9 Da
Crystallization: Crystals were grown using the hanging-drop vapor diffusion technique. Two microliter drops containing equal volumes of protein solution and a buffer solution composed of 200 mM MgCl2, 17% PEG 3350, 100 mM Bis-Tris, pH 5.5 were equilibrated against a well containing 300 µl of the buffer solution. Crystals reached a size of ~200 microns over a period of 3-7 days.
NMR Spectroscopy:
Data Collection: Resolution: 2.1 Å, X-ray source: Synchrotron SLS-X06, single wavelength.
Data Processing: