PTPN14

PDB:2BZL

Revision

Revision Type:created
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Entry Clone Accession:gi:34328899 NP_005392 .
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:

mhhhhhhssgvdlgtenlyfqsMVDATRVPMDERFRTLKKKLEEGMVFTEYEQIPKKKANGIFSTAALPENAERSRIREVVPYEENR
VELIPTKENNTGYINASHIKVVVGGAEWHYIATQGPLPHTCHDFWQMVWEQGVNVIAMVTAEEEGGRTKSHRYWPKLGSKHSSATYG
KFKVTTKFRTDSVCYATTGLKVKHLLSGQERTVWHLQYTDWPDHGCPEDVQGFLSYLEEIQSVRRHTNSMLEGTKNRHPPIVVHCSA
GVGRTGVLILSELMIYCLEHNEKVEVPMMLRLLREQRMFMIQTIAQYKFVYQVLIQFLQNSRLIDSKGGYGSE

Vector:pLIC-SGC1

Growth

Medium:
Antibiotics:
Procedure:1 mL 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. Binding buffer: 50mM HEPES pH 7.5; 500 mM NaCl; 5 mM imidazole, 5% glycerol.

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 and then washed with 20 mL binding buffer prior to loading the sample.

Buffers: 50mM HEPES pH 7.5; 500 mM NaCl; 5 mM imidazole, 5% glycerol; 0.5 mM TCEP

Procedure: 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.

Buffers: Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 0.5 mM TCEP. Wash buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM Imidazole, 5% glycerol, 0.5 mM TCEP. Elution buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 50 to 250 mM Imidazole , 5% Glycerol, 0.5 mM TCEP.

Procedure: The flowthrough from column 1 was loaded by gravity flow on the Ni-NTA column. The column was then washed with 50 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.

Column 3: Size Exclusion Chromatography

Buffers: 50 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM TCEP

Procedure: TEV-cleaved PTPN14 was directly applied to a S200 16/60 HiLoad gel filtration column equilibrated in 50 mM HEPES pH 7.5, 500 mM NaCl, 0.5 mM TCEP 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:
Ligand
MassSpec:
Crystallization: Crystals were grown at 20°C in 200nl sitting drops mixing 150 nl of protein with 50 nl of a solution containing 0.1 M Bis-Tris HCl pH 5.0; 0.2 M Li2SO4 and 25% PEG 3350. The crystals were cryo-protected using 20% ethylene glycol which was added to the drop 30 seconds prior to mounting.
NMR Spectroscopy:
Data Collection:
Data Processing: