PTPN7

PDB:2A3K

Revision

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Entry Clone Accession:gi|18375658
Entry Clone Source: Purely Proteins Ltd.
SGC Clone Accession:
Tag:N-terminal histag with TEV cleavage site: mhhhhhhssgvdlgtenlyfq*s(m)
Host:BL21 (DE3)R3 (Phage resistant strain)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmNTPREVTLHFLRTAGHPLTRWALQRQPPSPKQLEEEFLKIPSNFVSPEDLDIPGHASKDRYKTI
LPNPQSRVCLGRAQSQEDGDYINANYIRGYDGKEKVYIATQGPMPNTVSDFWEMVWQEEVSLIVMLTQLREGKEKCVHYWPTEEETY
GPFQIRIQDMKECPEYTVRQLTIQYQEERRSVKHILFSAWPDHQTPESAGPLLRLVAEVEESPETAAHPGPIVVHCSAGIGRTGCFI
ATRIGCQQLKARGEVDILGIVCQLRLDRGGMIQTAEQYQFLHHTLALYAGQLPEEP

Vector:pNIC-SGC

Growth

Medium:
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.

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, 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: 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.

Enzymatic treatment: (His tag cleavage using TEV) Samples containing PTPN7 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 using the following procedure. The buffer was changed to 50 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM TCEP using a 10 kDa cut-off concentrator and the TEV-treated protein sample mixed with Ni-NTA agarose for 30 minutes at 4°C. The resin was centrifuged and the supernatant containing the cleaved protein collected.

Column 3: Size Exclusion Chromatography. Buffers: 50 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM TCEP. Procedure: TEV-cleaved PTPN7 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 150 nl sitting drops mixing 100 nl of protein with 50 nl of a solution containing 0.1M Tris HCl pH 8.5; 2.0 M Ammonium dihydrogen phosphate
NMR Spectroscopy:
Data Collection:
Data Processing: