PTPN3

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Revision

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Entry Clone Accession:gi|18104986
Entry Clone Source: Origene
SGC Clone Accession:
Tag:
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

MDTLEGSMAQLKKGLESGTVLIQFEQLYRKKPGLAITFAKLPQNLDKNRYKDVLPYDTTRVLLQGNEDYINASYVNMEIPAANLVNK
YIATQGPLPHTCAQFWQVVWDQKLSLIVMLTTLTERGRTKCHQYWPDPPDVMNHGGFHIQCQSEDCTIAYVSREMLVTNTQTGEEHT
VTHLQYVAWPDHGVPDDSSDFLEFVNYVRSLRVDSEPVLVHCSAGIGRTGVLVTMETAMCLTERNLPIYPLDIVRKMRDQRAMMVQT
SSQYKFVCEAILRVYEEGLVQM

Vector:pLIC-SGC1

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. 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.

Enzymatic treatment: (His tag cleavage using TEV) Samples containing PTPN3 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: SEC

Buffers: 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM DTT.

Procedure: AKTA-prime

Protein concentration: Centricons 30 kDa cut off

Extraction

Procedure
Frozen pellets were thawed and cells lysed using a high pressure cell disrupter. The lysate was centrifuged at 17,000 rpm for 40 minutes and the supernatant collected for purification.
Concentration:
Ligand
MassSpec:
Crystallization:PTPN3 was crystallized using the vapor diffusion method at 20°C and a protein concentration of 7.5 mg/ml. 150nl of the protein was mixed with 50nl of a solution containing 2 M (NH4)2SO4 0.1 MBIS-TRIS 5.5. Crystals grew within 3 days and were cryo-stabilize using the crystallization solution and 30% sucrose.
NMR Spectroscopy:
Data Collection:
Data Processing: