PTPN4

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Revision

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SGC Clone Accession:
Tag:mhhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag.
Host:BL21 (DE3)R3 (Phage resistant strain)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmEEKLENEPDFQYIPEKAPLDSVHQDDHSLRESMIQLAEGLITGTVLTQFDQLYRKKPGMTMSCA
KLPQNISKNRYRDISPYDATRVILKGNEDYINANYINMEIPSSSIINQYIACQGPLPHTCTDFWQMTWEQGSSMVVMLTTQVERGRV
KCHQYWPEPTGSSSYGCYQVTCHSEEGNTAYIFRKMTLFNQEKNESRPLTQIQYIAWPDHGVPDDSSDFLDFVCHVRNKRAGKEEPV
VVHCSAGIGRTGVLITMETAMCLIECNQPVYPLDIVRTMRDQRAMMIQTPSQYRFVCEAILKVYEEGFVKPLTTSTNKLT

Vector: Vector: pNIC28-Bsa4

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; 0.5 mM TCEP.

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.
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.
The flow through 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 and 250 mM); fractions were collected until essentially all protein was eluted.

Enzymatic treatment: (His tag cleavage using TEV) Samples containing PTPN4 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 after buffer exchange to 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP using a 10 kDa cut-off concentrator .

Column 3: Size Exclusion Chromatography. Superdex S200 16/60 HiLoad
TEV-cleaved PTPN4, after re-binding to NiNTA, was directly applied to a S200 16/60 HiLoad gel filtration column equilibrated in 50 mM HEPES pH 7.5, 300 mM NaCl, 10 mM DTT using either an ÄKTAprime or ÄKTAxpress system

Protein concentration: Protein was concentrated to 7.5 mg/ml using an Amicon 10 kDa cut-off concentrator

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:LC- ESI -MS TOF confirmed the correct mass expected for this construct. Predicted mass of TEV-cleaved protein = 36643; Measured = 36643
Crystallization: Crystals were grown at 20°C in 300 nl sitting drops mixing 150 nl of protein with 150 nl of a solution containing 0.1M HEPES pH 6.8, 0.1 M NaCl and 1.3 M (NH4)SO4. The crystals grew as long needles and were cryo-protected using 20% ethylene glycole which was added to the drop 30 seconds prior to mounting and flash flash freezing in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Resolution: 2.45 Å. X-ray source: Diffraction data were collected at the SLS beamline X10 at a single wavelength (0.979 Å).
Data Processing: