PTPN22: Human protein tyrosine phosphatase, non-receptor type 22

PDB:2P6X

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NM_015967; gi|7706280
Entry Clone Source: Origene
SGC Clone Accession:PTPN22A-c311
Tag: Tag sequence: Non-cleavable C-terminal His6 tag.
Host:BL21 (DE3) Rosetta

Construct

Prelude:NM_015967: 84 bp deletion from 1942 to 2025 of ORF, mutation at 1858 changes the aa from Trp to Arg. These mutations are outside the catalytic domain and are not part of the determined crystal structure
Sequence:

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MDQREILQKFLDEAQSKKITKEEFANEFLKLKRQSTKYKADKTYPTTVAEKPKNIKKNRYKDILPYDYSRVELSLITSDEDSSYINA
NFIKGVYGPKAYIATQGPLSTTLLDFWRMIWEYSVLIIVMACMEYEMGKKKCERYWAEPGEMQLEFGPFSVSCEAEKRKSDYIIRTL
KVKFNSETRTIYQFHYKNWPDHDVPSSIDPILELIWDVRCYQEDDSVPICIHCSAGCGRTGVICAIDYTWMLLKDGIIPENFSVFSL
IREMRTQRPSLVQTQEQYELVYNAVLELFKRQMDVIRDKHSahhhhhh
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Vector:pNIC-CH

Growth

Medium:
Antibiotics:
Procedure: Transformed 50 µl competent BL-21 (DE3) phage resistant cells with 10 µl of the plasmid DNA and plated out onto LB plate plus 50 µg/ml kanamycin. The next day colonies were picked out into fresh deep well blocks containing 1 ml TB + 50 µg/ml kanamycin which were grown overnight and glycerol stocks were prepared by adding 333 ml of 60 % glycerol to 1 ml of cell suspension, which were stored at -80°C to be used for future scale up preparations. The glycerol stock was used to inoculate 10 ml of TB (Terrific Broth) supplemented with 50 µg/ml kanamycin. This starter culture was grown overnight at 37°C and used to inoculate a 1 liter culture in the same medium. The culture was grown at 37°C until the OD600 reached ~2.0 . After that the temperature was lowered to 18°C. Protein production was induced with 1mM IPTG and recombinant PTPN22 was expressed at that temperature over night. The next day cells were harvested by centrifugation at 4000 rpm for 15 minutes. The cell pellet was stored at -80°C degrees.

Purification

Procedure
All purification steps were carried out using an AKTAexpress system (GE Healthcare) at 7degC. The lysate was loaded on a pre-equilibrated His-trap column at 0.8 ml/min, using a standard purification method. After loading, the column was washed at 0.8 ml/min with 10 ml binding buffer, then 20 ml wash buffer, and protein was eluted with 5 ml of elution buffer. The peak fraction was collected automatically according to A280.

The PTPN22 containing fraction eluted of the Ni-affinity chromatography was automatically loaded on the SEC column at 1.2 ml/min. PTPN22 eluted at a retention time corresponding to the monomeric protein. Eluted fractions were 95% pure as judged by SDS-PAGE.

Protein concentration: 12 mg/ml in SEC buffer using a centricon with a 10kDa cut off

Extraction

Procedure
The cell pellet (38 g) was re-suspended in one volume (38 ml) of 2x extraction buffer. The re-suspended cells were lysed by one passage through a Constant Systems cell breaker and subsequent sonication; the cell breaker was washed with 1x extraction buffer, bringing the total volume to 120 ml. DNA was precipitation by addition of PEI to a final concentration of 0.15 % during an incubation time of 30 min on ice, followed by a centrifugation at 17,000 rpm (4°C); The supernatant was further cleared by filtration through a 0.2 µm serum Acrodisc filter.
Concentration:
Ligand
MassSpec:ESI-MS revealed that the protein had the expected mass of 36462 Da.
Crystallization: The PTPN22 was crystallized at 20degC using the sitting-drop vapor diffusion method. 100nanoL of concentrated protein was mixed with 100nl of well solution and equilibrated with 100 µl of the well solution. Diffraction quality crystals were obtained in a solution containing 100 mM Bicine pH 7.9 and 25% PEG 10K.
NMR Spectroscopy:
Data Collection: Crystals were cryoprotected using 25% ethylene glycol and flash frozen in liquid nitrogen. Diffraction data were collected to 1.8Å at the Swiss light source beam-line X10SA at a single wavelength of 0.979Å.
Data Processing: