PTPN18 - Human tyrosine-protein phosphatase non-receptor type 18

PDB:2OC3

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|18375655
Entry Clone Source: Purely Proteins
SGC Clone Accession:
Tag: PreScissionÂ (rhinovirus 3C)- protease cleavable (*) GST tag:
mspilgywkikglvqptrllleyleekye hlyerdegdkwrnkkfelglefpnlpyyi dgdvkltqsmaiiryiadkhnmlggcpke raeismlegavldirygvsriayskdfet lkvdflsklpemlkmfedrlchktylngd hvthpdfmlydaldvvlymdpmcldafpk lvcfkkrieaipqidkylksskyiawplq gwqatfgggdhppksdlevlfq*gplgsp gip
Host:BL21(DE3) phage resistant

Construct

Prelude:
Sequence:

```
GPLGSPGIPDSARSFLERLEARGGREGAV LAGEFSDIQACSAAWKADGVCSTVAGSRP ENVRKNRYKDVLPYDQTRVILSLLQEE
GH SDYINGNFIRGVDGSLAYIATQGPLPHTL LDFWRLVWEFGVKVILMACREIENGRKRC ERYWAQEQEPLQTGLFCITLIKEK
WLNED IMLRTLKVTFQKESRSVYQLQYMSWPDRG VPSSPDHMLAMVEEARRLQGSGPEPLCVH CSAGCGRTGVLCTVDYVRQLL
LTQMIPPD FSLFDVVLKMRKQRPAAVQTEEQYRFLYH TVAQMFCSTLQNA
```

Vector:pGEX-6P2

Growth

Medium:
Antibiotics:
Procedure: Starter cultures from freshly transformed colonies in 10 ml LB, and ampicillin were grown overnight. This was diluted 1:1000 in fresh media (6L) and was grown at 37°C to an OD600 of 0.3 and than transferred to 18°C. Expression was induced at an OD600 of 0.8 using 1 mM IPTG. Cells were harvested after 3h by centrifugation, transferred to 50-ml tubes, and frozen in liquid nitrogen.

Purification

Procedure
Column 1: Glutathione Sepharose 4B affinity, 5 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer.

Procedure: Supernatant was applied at gravity flow, followed by a wash with 30 ml binding buffer. The GST-fusion was cleaved while bound to the column by addition of PreScission protease. The column was gently rotated overnight a 4°C then protein eluated with 3 bed volumes of binding buffer.

Column 3: SEC
Procedure: AKTA-prime

Protein concentration: Centricons 10 kDa cut off

Extraction

Procedure
The cell pellets (20 g wet wt) were re-suspended in 50 ml extraction buffer, and lysed by a high pressure cell disrupter. Supernatant was centrifuged for 30 minutes at 60,000 rpm.
Concentration:
Ligand
MassSpec:LC-ESI-MStof confirmed the correct mass expected for this construct. Expected mass, 34254; Observed mass, 34255
Crystallization: Crystals were obtained using sitting drop method at 4°C. Drops were prepared using 150 nl of protein (8 mg/ml concentration) and 150 nl of the well solution (0.1M HEPES pH 6.8, 25% PEG-3350 and 6% Jeffamine M-600).
NMR Spectroscopy:
Data Collection: Resolution: 1.5 Å; X-ray source: Synchrontron SLS-X10.
Data Processing: