PTPRB

PDB:2AHS

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|18491010; NP_002828
Entry Clone Source: Purely Proteins
SGC Clone Accession:
Tag: PreScission (rhinovirus 3C)- protease cleavable (*) GST tag:
mspilgywkikglvqptrllleyleekyehlyerdegdkwrnkkfelglefpnlpyyidgdvkltqsmaiiryiadkhnmlggcpke
raeismlegavldirygvsriayskdfetlkvdflsklpemlkmfedrlchktylngdhvthpdfmlydaldvvlymdpmcldafpk
lvcfkkrieaipqidkylksskyiawplqgwqatfgggdhppksdlevlfq*gplgspgip
Host:E. coli Rosetta strain

Construct

Prelude:
Sequence:

```
GPLGSPGIPNQFEGHFMKLQADSNYLLSKEYEELKDVGRNQSCDIALLPENRGKNRYNNILPYDATRVKLSNVDDDPCSDYINASYI
PGNNFRREYIVTQGPLPGTKDDFWKMVWEQNVHNIVMVTQCVEKGRVKCDHYWPADQDSLYYGDLILQMLSESVLPEWTIREFKICG
EEQLDAHRLIRHFHYTVWPDHGVPETTQSLIQFVRTVRDYINRSPGAGPTVVHCSAGVGRTGTFIALDRILQQLDSKDSVDIYGAVH
DLRLHRVHMVQTECQYVYLHQCVRDVLRARKLRS
```

Vector:pGEX-6P2

Growth

Medium:
Antibiotics:
Procedure: Starter cultures from freshly transformed colonies in 10 ml LB, 0.1 mg/ml choloramphenicol and ampicilline were grown overnight. This was diluted 1:1000 in fresh media (6L) and was grown at 37°C to a 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.

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

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 2: Ion exchange Mono Q column .

Buffers: A: 50 mM Hepes pH 7.5>. B: 50 mM Hepes pH 7.5, 1000 mM NaCl.

Procedure: The partially purified protein was applied to MonoQ in buffer A and eluted from the column by a linear gradient.

Column 3: SEC

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

Procedure: AKTA-prime

Protein concentration: Centricons 10 kDa cut off

Extraction

Procedure
Extraction buffer: 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM DTT. 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:
Crystallization: Crystals were obtained using sitting drop method at 4°C. Drops were prepared using 500 nl of protein (8 mg/ml mg/ml concentration) and 500 nl of the well solution (0.1M Hepes pH 7, 9% PEG 6000, 8% Ethylene glycol).
NMR Spectroscopy:
Data Collection:
Data Processing: