PTPRR

PDB:2A8B

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source: Purely Protein Ltd.
SGC Clone Accession:
Tag: Tag sequence:
mspilgywkikglvqptrllleyleekyehlyerdegdkwrnkkfelglefpnlpyyidgdvkltqsmaiiryiadkhnmlggcpker
aeismlega
vldirygvsriayskdfetlkvdflsklpemlkmfedrlchktylngdhvthpdfmlydaldvvlymdpmcldafpklvcfkkrieaip
qidkylksskyia wplqgwqatfgggdhppksdlevlfq*gplgsp gip

PreScission (rhinovirus 3C)- protease cleavable (*) GST tag
Host:E.coli BL21 (DE3)

Construct

Prelude:
Sequence:

```
mspilgywkikglvqptrllleyleekyeehlyerdegdkwrnkkfelglefpnlpyyidgdvkltqsmaiiryiadkhnmlggcpk
eraeismlegavldirygvsriayskdfetlkvdflsklpemlkmfedrlchktylngdhvthpdfmlydaldvvlymdpmcldafp
klvcfkkrieaipqidkylksskyiawplqgwqatfgggdhppksdlevlfqgplgspgipSRILTRSQLRDVVASSHLLQSEFMEI
PMNFVDPKEIDIPRHGTKNRYKTILPNPLSRVCLRPKNVTDSLSTYINANYIRGYSGKEKAFIATQGPMINTVDDFWQMVWQEDSPV
IVMITKLKEKNEKCVLYWPEKRGIYGKVEVLVISVNECDNYTIRNLVLKQGSHTQHVKHYWYTSWPDHKTPDSAQPLLQLMLDVEED
RLASQGRGPVVVHCSAGIGRTGCFIATSIGCQQLKEEGVVDALSIVCQLRMDRGGMVQTSEQYEFVHHALCLYESRLSAET
```

Vector:pGEX-6P2

Growth

Medium:
Antibiotics:
Procedure: Medium: Starter cultures from freshly transformed colonies in 10 mL LB, 0.1 mg/mL ampicillin were grown overnight. This was diluted 1:1000 in fresh media (6L) and was grown at 37 o C to a OD 600 of 0.3 and than transferred to 18 o C. Expression was induced at an OD 600 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

Extraction

Procedure
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 20,000 rpm in a JA 20 rotor.
Concentration:
Ligand
MassSpec:
Crystallization: Protein concentration, 10 mg/mL. Well solution: 150 mM Na malate, pH 7.0; 20% PEG3350. Crystals were grown at 4 ºC in 200 nL sitting drops
NMR Spectroscopy:
Data Collection:
Data Processing: