PLRG1

PDB:4YVD

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:4505895
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQG
Host: Sf9

Construct

Prelude:
Sequence:

```
gTAPSGSEYRHPGASDRPQPTAMNSIVMETGNTKNSALMAKKAPTMPKPQWHPPWKLYRVISGHLGWVRCIAVEPGNQWFVTGSADR
TIKIWDLASGKLKLSLTGHISTVRGVIVSTRSPYLFSCGEDKQVKCWDLEYNKVIRHYHGHLSAVYGLDLHPTIDVLVTCSRDSTAR
IWDVRTKASVHTLSGHTNAVATVRCQAAEPQIITGSHDTTIRLWDLVAGKTRVTLTNHKKSVRAVVLHPRHYTFASGSPDNIKQWKF
PDGSFIQNLSGHNAIINTLTVNSDGVLVSGADNGTMHLWDWRTGYNFQRVHAAVQPGSLDSESGIFACAFDQSESRLLTAEADKTIK
VYREDDTATEETHPVSWKPEIIKRKRF
```

Vector:pFBOH-MHL

Growth

Medium:
Antibiotics:
Procedure:PLRG1 was expressed in Sf9 cells. Sf9 cells were infected with P3 viral stocks and incubated at 27˚C, 100 rpm for 48-72 hours, until the cell viability dropped to 70-80%.

Purification

Procedure
The clarified lysate was loaded onto a 2 mL TALON column (Clonetech). The column was washed with 50 column volumes of 20 mM HEPES buffer, pH 7.4, containing 500 mM NaCl, 5% cglycerol and 5 mM imidazole, and the protein was eluted with elution buffer (20 mM HEPES, pH 7.4, 500 mM NaCl, 5% glycerol, 250 mM imidazole). The eluted protein was loaded on a Superdex200 column (GE Healthcare), equilibrated with 20 mM PIPES buffer, pH 6.5, and 250 mM NaCl. Pooled fractions containing PLRG1 were subjected to TEV treatment to remove His-tag. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30S column (10x10) (GE Healthcare), equilibrated with buffer containing 20 mM PIPES, pH 6.5, and eluted with linear gradient of NaCl up to 500 mM concentration (20 CV).

Enzymatic treatment: TEV cleavage.

Extraction

Procedure
Cells were harvested by centrifugation at 4,000 rpm, 4 °C for 15 minutes. The cell pellets were washed one time in cold 1X PBS buffer, and frozen in liquid nitrogen and stored at -80˚C. For purification the frozen cell paste was thawed and resuspended in lysis buffer containing 20 mM HEPES, pH 87.4, 500 mM NaCl, 5 mM imidazol, 2 mM β-mercaptoethanol, 5% glycerol, 0.6% NP-40, protease inhibitor cocktail (Roche), 3000 U of benzonase (Novagen). Cells were lyzed by brief sonication.
Concentration:7.7 mg/ml
Ligand
MassSpec:expected MW = 41669.2 Da, measured MW= 37625.74 Da.
Crystallization: Purified PLRG1 (7.6 mg/mL) was crystallized using the sitting drop vapor diffusion method at 20 °C by mixing 2 µl of the protein solution with 1 µl of the reservoir solution containing 20% PEG3350, 0.2 M CaCl2.
NMR Spectroscopy:
Data Collection:
Data Processing: