PPPDE1

PDB:3EBQ
Entry Clone Accession:NP_056519
Entry Clone Source:pppde1.LIFESEQ1602503.OBS.IHS1380-97433953.pINCY
SGC Clone Accession:pppde1.001.168.084B09 (SDC084B09)
Tag:N-terminal tag: MGSSHHHHHHSSGLVPR*GS (removed).
Host:*E.coli* BL21-CodonPlus(DE3)-V2R
Vector:pET28a-LIC

Sequence:

mgsshhhhhhssglvpr*gsMEPPNLYPVKLYVYDLSKGLARRLSPIMLGKQLEGIWHTSIVVHKDEFFFGSGGISSCPPGGTLLGP
PDSVVDVGSTEVTEEIFLEYLSSLGESLFRGEAYNLFEHNCNTFSNEVAQFLTGRKIPSYITDLPSEVLSTPFGQALRPLLDSIQIQ
PPGGSSVGRPNGQS

Growth

Procedure: The protein was expressed in E. coli BL21-CodonPlus(DE3)-V2R grown in Terrific Broth (TB) in the presence of 50 µg/mL of kanamycin at 37°C to an OD600 of 7.5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.05 mM, and incubated overnight at 15°C. The culture was centrifuged and the cell pellets were collected and stored at -80°C.

Purification

Procedure: Imac: The cleared lysate from a 4 L culture was loaded onto 3 ml TALON metal-affinity resin column (BD Biosciences) at 4ºC. The column was washed with 40 ml Wash buffer, and the protein was eluted with 10 ml Elution buffer.

Tag removal: 1 Unit of thrombin (Sigma T9681) per milligram of protein was added to the 10 mL sample, stored overnight without shaking at 4ºC.

Gel-filtration: Protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with Gel Filtration buffer and concentrated to 30 mg/ml by ultrafiltration using Amicon Ultra centrifugal filter with 10 kD cutoff and stored at -80ºC.

Protein yield was 15 mg per liter of bacterial culture.

Extraction

Procedure: The cell pellet was defrosted and cells were lysed by sonication, 10 s on, 10 s off at 40% amplitude for 10 min. The lysate was cleared by centrifugation for 45 minutes at 15,500 RPM, 4°C.

Structure Determination

MassSpec: Mass-spectroscopy by LCMS shows that the product was pure and correct molecular weight.

Crystallization: Purified protein was crystallized using the hanging drop vapor diffusion method. Crystals grew when the protein (30 mg/mL) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop was equilibrated against a reservoir solution containing 10% PEG 4000, 0.1 M Sodium acetate, and 0.1 M Tris HCl at pH 4.6 in 293 Kelvin.