

PPT1

PDB:3GRO

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:BC008426

Entry Clone Source:MGC, AU44-G2,

SGC Clone Accession:PPT1:DCC001-D04:C44672

Tag:C-terminal tag: EFVEHHHHHHHH

Host:Sf9 insect cells

Construct

Prelude:

Sequence:

ARALQHLDPPAPLPLVIWHGMGDSCCNPLSMGAIKKMVEKKIPGIYVLSLEIGKTLMEDVENSFFLNVNSQVTTVCQALAKDPKLQQ
GYNAMGFSQGGQFLRAVAQRCPSPPMINLISVGGQHQQVFGLPRCPGESSHICDFIRKTLNAGAYSKVVQERLVQAEYWHDPKEDV
YRNHSIFLADINQERGINESYKKNLMAKKFVMVKFLNDSIVDPVDSEWFGFYRSGQAKETIPLQETSLYTQDRLGLKEMDNAGQLV
FLATEGDHLQLSEEWFYAHIIIPFLG

Vector:pFHMSP-LIC-C

Growth

Medium:

Antibiotics:

Procedure:Plasmid transfer vector pFHMSP-LIC-C containing the gene was transformed into DH10Bac E.coli cells (Invitrogen) to obtain recombinant viral DNA. SF9 cells were transfected with Bacmid DNA using Cellfectin reagent (Invitrogen), and recombinant baculovirus was generated. Viral stock was amplified from P1 to P3.

Sf9 cells grown in HyQ® SFX Insect Serum Free Medium (Cat.# SH3027802) at density of 3 million cells per milliliter of media and with viability not less than 97% were infected with 7 mL of P3 viral stock for each 1 L of cell culture. Cell culture medium was collected after 4 days of incubation on a shaker at 100 RPM and 27 °C when cells viability dropped to 25-45%.

Purification

Procedure

IMAC purification: A 1.6 L volume of medium was mixed with 20 mL pre-equilibrated NiNTA Superflow beads and stirred (Talboys/Troemner) for 1 hour. The resin was transferred to a 50 mL

gravity column, washed with 50 mL of Washing Buffer, and the protein was eluted with 10 mL of Elution Buffer. A second round of NiNTA batch absorption has been performed for increased protein yield.

Bound protein was eluted from the IMAC columns with Elution Buffer and loaded onto the Gelfiltration (GF) column. The chromatogram from gel filtration showed one major protein peak that consisted of PPT1 confirmed by SDS-PAGE analysis.

Extraction

Procedure

The cultured medium was centrifuged at 14,000 xg for 15 minutes, and the pH of the supernatant was adjusted to 7.5 at room temperature by adding 10x Buffer_A. Protease inhibitors were added to final concentrations of 1 mM phenylmethanesulfonyl fluoride (PMSF, Bioshop) and 2 mM benzamidine hydrochloride (Sigma).

Concentration: Purified protein was concentrated using 15 mL concentrators with an appropriate molecular weight cut-off (Amicon Ultra-15 10,000 MWCO, Millipore) to a final value of 10 mg/mL. Average yield was about 2.5 mg/L.

Ligand

MassSpec:

Crystallization: Crystallization was setup using sitting drops with Red Wings and SGC-I screens initially. Small crystals were seen at condition: 0.1M NaCacodylate, pH 5.50, 25% PEG3350, 0.2M (NH₄)₂SO₄, protein concentration 10 mg/mL. Crystal used for structure determination were grown in: 0.1M NaCacodylate, pH 5.40, 23.64% PEG3350, 0.2M (NH₄)₂SO₄, protein concentration 10 mg/mL. Cryoprotectant used 0.9V well solution plus 0.1V 80% Glycerol.

NMR Spectroscopy:

Data Collection:

Data Processing: