Tb10.389.0330

PDB:3GUE

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession: Tb10.389.0330
Entry Clone Source:
SGC Clone Accession: Tb10.389.0330:MAC041-B07
Tag:N-terminal tag: mhhhhhhssgrenlyfqg
Host:BL21(DE3)-V2R-pRARE2

Construct

Prelude:
Sequence:

LNPPSAFSGAALACLEKMQASGVEEKCIHIFLIQHALVRKGETGYIPEKSISPVESLPFLQGIETKGENTALLRQAVVLKLNGGLGT
GMGLNGPKSLLQVKNGQTFLDFTALQLEHFRQVRNCNVPFMLMNSFSTSGETKNFLRKYPTLYEVFDSDIELMQNRVPKIRQDNFFP
VTYEADPTCEWVPPGHGDVYTVLYSSGKLDYLLGKGYRYMFISNGDNLGATLDVRLLDYMHEKQLGFLMEVCRRTESDKKGGHLAYK
DVIDETTGQTRRRFVLRESAQCPKEDEDSFQNIAKHCFFNTNNIWINLMELKKMMDEQLGVLRLPVMRNPKTVNPQDSQSTKVYQLE
VAMGAAISLFDRSEAVVVPRERFAPVKTCSDLLALRSDAYQVTEDQRLVLCEERNGKPPAIDLDGEHYKMIDGFEKLVKGGVPSLRQ
CTSLTVRGLVEFGADVSVRGNVVIKNLKEEPLIIGSGRVLDNEVVVVE

Vector:p15-mhl

Growth

Medium:TB
Antibiotics:
Procedure: Tb10.389.0330:B7 was expressed in E. coli BL21(DE3)-V2R-pRARE2 cells in Terrific Broth (TB) in the presence of ampicillin/chloramphenicol (50 microg/mL and 25 microg/mL respectively). A single colony was inoculated into 10 mL of LB with of ampicillin/chloramphenicol (50 microg/mL and 25 microg/mL respectively) in a 50 mL Falcon tube and incubated with shaking at 250 rpm overnight at 37 °C. The culture was transferred into 50 mL of TB with 50 microg/mL ampicillin in a 250 mL shaking flask and incubated at 37 °C for 3 hours. Then the culture was transfered into 1.8 L of TB with 50 microg/mL kanamycin and 0.3 mL of antifoam (Sigma) in a 2 L bottle and cultured using the LEX system to an OD 600 of ~5, cooled to 15 °C, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 °C.

Purification

Procedure

STEP1:The cleared lysate was loaded onto a column prepacked with 10 g DE52 (Whatman) anion exchange resin (previously activated with 2.5 M NaCl and equilibrated with Binding Buffer); and subsequently onto a 3 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1 - 1.5 mL/min. The volume of the Ni-NTA resin was pre-determined by the predicted protein yield from test expression analysis. After the lysate was loaded, the DE52 was further washed with 20 mL of Binding Buffer. The Ni-NTA column was then washed with 200 mL of Wash Buffer at 2 - 2.5 mL/min. After washing, the protein was eluted with 15 mL of Elution Buffer. EDTA was immediately added to the elution fraction to 1 mM; and TCEP was added to 1 mM after approximately 15 more minutes.

STEP2:(Cut His Tag) Added TEV (with activity 1:50 and concentration of 12mg/mL) to the protein and dialysis in 10mM HEPES, 500mM NACL, 5mM Imidazole, and 5mM DTT overnight. The day after run mass spectroscopy to make sure His Tag completely cut and then pass through Ni-NTA, and filterated with syringe driven filter unite(0.22um) for running Gel filtration.

STEP3: The sample was loaded onto a Sephadex S200 26/60 gel filtration column pre-equilibrated with 10 mM HEPES, pH 7.5 and 500 mM NaCl, 5mMDTT. The collected fractions corresponding to the correct eluted protein peak were concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). The protein sample identity were evalulated by mass spectroscopy. The concentrated sample (18 mg/mL) was stored at -80 ºC.

Extraction

Procedure
The culture was harvested by centrifugation. Pellets from 4 L of culture were resuspended to approximately 40 mL/L of cell culture in Binding Buffer with the addition of protease inhibitors (1 mM benzamidine and 1 mM phenylmethyl sulfonyl fluoride (PMSF)). Resuspended pellets stored at -80 ºC were thawed overnight at 4 ºC on the day before purification. Prior to mechanical lysis, each pellet from 1 L of culture was pretreated with 0.5 % CHAPS and 500 units of benzonase for 30 minutes at room temperature. Cells were mechanically lysed with a microfluidizer (Microfluidizer Processor, M-110EH) at approximately 18000 psi; and the cell lysate was centrifuged using a Beckman JA-25.50 rotor at ~75000 x g (24000 rpms) for 20 minutes at 10 ºC.
Concentration:
Ligand
MassSpec:
Crystallization: The protein was crystallized at 20 ºC in 23% w/v PEG 3350, 0.1 M Ammonium Sulphate, 0.1M Bis-Tris pH - 5.5, 18% Glycerol using the Hanging drop vapor diffusion method. Ligands10mM UDPGlucose, 2mm MgCl2 added to protein before setting up plate
NMR Spectroscopy:
Data Collection:
Data Processing: