EPB41L3(DAL-1)

PDB:2HE7

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC006141
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*s(m).
Host:

Construct

Prelude:
Sequence:

MHHHHHHSSGVDLGTENLYFQSMKPKSMQCKVILLDGSEYTCDVEKRSRGQVLFDKVCEHLNLLEKDYFGLTYRDAENQKNWLDPAK
EIKKQVRSGAWHFSFNVKFYPPDPAQLSEDITRYYLCLQLRDDIVSGRLPCSFVTLALLGSYTVQSELGDYDPDECGSDYISEFRFA
PNHTKELEDKVIELHKSHRGMTPAEAEMHFLENAKKLSMYGVDLHHAKDSEGVEIMLGVCASGLLIYRDRLRINRFAWPKVLKISYK
RNNFYIKIRPGEFEQFESTIGFKLPNHRAAKRLWKVCVEHHTFFRLLLPEAPPK

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Cells from glycerol stocks were grown in 20 mL of TB supplemented with 8 g/L glycerol, 50µg/mL Kanamycin at 30°C over night. The following morning 20 mL of the over night cultures were used to inoculate 1500 mL Terrific Broth with 8 g/L 87 % glycerol, 50 µg/mL Kanamycin, 100 µL BREOX (anti-foaming agent) in glass flasks in the Large Scale Expression System (LEX). Cells were grown at 37°C until OD600 of 2.5. The cultivations were down-tempered to 18 °C for 1h in water bath. Expression of target protein was induced by addition of 0.5 mM IPTG and was allowed to continue over night at 18 °C.

Purification

Procedure
50 mM Sodium-Phosphate, pH 7.5, 10 mM Imidazole, 500 mM NaCl, 10% glycerol, 0.5 mM TCEP (IMAC Bind/Wash1 Buffer); 50 mM Sodium-Phosphate, pH 7.5, 25 mM Imidazole, 500 mM NaCl, 10% glycerol, 0.5 mM TCEP (IMAC Wash2 Buffer); 50 mM Sodium-Phosphate, pH

7.5, 500 mM Imidazole, 500 mM NaCl, 10% glycerol, 0.5 mM TCEP (IMAC Elution Buffer); 20 mM HEPES pH 7.5, 300 mM NaCl, 10% glycerol, 0.5 mM TCEP (Gelfiltration Buffer). Columns: HiTrap Chelating 1 mL (IMAC); HiLoadÂ 16/60 Superdex 200 Prep Grade (Gel filtration), all from GE Healthcare. Purification was conducted automatically on an ÄKTA-Xpress system (GE Healthcare) operated by UNICORN software at a flow of 0.8 mL/min. Prior to purification columns were equilibrated with IMAC Bind/Wash1 Buffer (HisTrap HP) and Gel filtration buffer (Superdex 200). The protein sample was loaded on the HisTrap HP column that was washed with IMAC Bind/Wash1 Buffer followed by IMAC Wash2 Buffer. Bound protein was eluted from the IMAC columns with 7.5 mL of IMAC Elution Buffer and loaded onto the Gel filtration column. The chromatogram from gel filtration showed one major protein peak that consisted of highly pure EPB41L3 as shown by SDS-PAGE analysis. TCEP was added to the pooled protein peak to a final concentration of 2 mM and betain was added to a final concentration of 0.5 M.The protein was concentrated to 44.7 mg/mL and flash frozen in small aliquots for storage at -80ºC. Yield of pure protein per litre of culture was 30 mg.

Extraction

Procedure

Concentration:
Ligand
MassSpec:
Crystallization:EPB41L3 crystallized in 20% ethanol and 10 mM Tris (pH 8.2). Needles (30x50x500 micron) grew within one day and diffracted to 2.5 Å at the ESRF beam line ID-23.1.
NMR Spectroscopy:
Data Collection:
Data Processing: