ECH1

PDB:2VRE

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:IMAGE:4300082
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal TEV-cleavable (at *) his-tag with the following sequence:mhhhhhhssgvdlgtenlyfq*s
Host:BL21(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*SMAPDHSYESLRVTSAQKHVLHVQLNRPNKRNAMNKVFWREMVECFNKISRDADCRAVVISGAGK
MFTAGIDLMDMASDILQPKGDDVARISWYLRDIITRYQETFNVIERCPKPVIAAVHGGCIGGGVDLVTACDIRYCAQDAFFQVKEVD
VGLAADVGTLQRLPKVIGNQSLVNELAFTARKMMADEALGSGLVSRVFPDKEVMLDAALALAAEISSKSPVAVQSTKVNLLYSRDHS
VAESLNYVASWNMSMLQTQDLVKSVQATTENKELKT

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Growth medium, induction protocol: TB - 10µl of overnight culture was added into 1L TB with 50µg/ml Kanamycin and 34µg/ml of Chloramphenicol. The cells were cultured at 37°C until the OD reached 1.372 and then decreased the temperature to 18°C. IPTG was added at 0.1mM (final concentration) and kept the culture at 18°C for overnight.

Purification

Procedure
Column 1: Ni-NTA Buffers: Binding buffer: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 5 mM Imidazole; Washing Buffer: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 40 mM Imidazole; Elution Buffer I: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 60 mM Imidazole; Elution Buffer II: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 80 mM Imidazole; Elution Buffer III: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 125 mM Imidazole; Elution Buffer VI: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 250 mM

Imidazole.Procedure: The column was packed by 6 ml of Ni-NTA slurry and equilibrated with 15 ml of binding buffer. The supernatant was loaded onto the column and the flow through was collected. The column was washed with 2x20 ml of of washing buffer. The protein was eluted with 5 ml of elution buffer I, II & III respectively and then 8 ml of elution buffer VI.Column 2: Superdex 200 Hiload 16 60Buffers: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 0.5 mM TCEP. Procedure: AKTA Purifier was used and run at 4°C. Fractions were analyzed by SDS - PAGE and the most purified fractions were collected.

Extraction

Procedure
Extraction buffer, extraction method: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 5 mM Imidazole. The cells were harvested by centrifugation at 4,000 g for 10 min. The pellet from 1 L culture was resuspended in 25 ml of extraction buffer. The sample was homogenized by using the EmulsiFlex-05 homogenizer (Glen Creston) and then centrifuged at 37505 g for 1hour and 15 min. The supernatant was kept for further purification.
Concentration:25mg/ml
Ligand
MassSpec: Mass spec characterization: 32741
Crystallization: Crystallisation: Crystals were grown by vapour diffusion at 20°C in 150 nl sitting drops. The drops were prepared by mixing 100 nl of protein solution and 50 nl of the reservoir solution comprised 18% PEG 8K, 0.2 M calcium acetate dihydrate and 0.1 M sodium cacodylate, pH 6.5. Crystals were transferred to a cryo-protectant consisting of 20% glycerol, 80 % well solution before flash-cooling in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Data Collection: Resolution: 2.0Å; X-ray source: SLS beam X10SA.
Data Processing: