THEM2

PDB:2H4U

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC000894
Entry Clone Source:
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*s(m).
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

MHHHHHHSSGVDLGTENLYFQSMRNFERVLGKITLVSAAPGKVICEMKVEEEHTNAIGTLHGGLTATLVDNISTMALLCTERGAPGV
SVDMNITYMSPAKLGEDIVITAHVLKQGKTLAFTSVDLTNKATGKLIAQGRHTKHLGN

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
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 75). 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 THEM2 as shown by SDS-PAGE analysis. TCEP was added to the pooled protein peak to a final concentration of 2 mM. The protein was concentrated to 12 mg/mL and flash frozen in small aliquots for storage at -80ºC. Yield of pure protein per litre of culture was 11 mg.

Extraction

Procedure
Cells were harvested by centrifugation (WCW 17 g) and pellets were resuspended in 70 mL of lysis buffer. After adding 8 µL of a 250 U/µl benzonase (Novagen) stock solution cells were disrupted by high pressure homogenization with a high-pressure homogenizer (Stansted) (2 passes) prior to centrifugation for 20 min at 49000 g in a Sorvall SS-34 rotor. The soluble fraction was decanted and filtered through a 0.20 µm filter.
Concentration:
Ligand
MassSpec:
Crystallization:THEM2 crystallized in 17% PEG 3350 and 0.2 M Na/K phosphate (pH 6.7). Rods (40x200 micron) grew within one day and diffracted to 2.2 Å at the ESRF beam line BM-14.
NMR Spectroscopy:
Data Collection:
Data Processing: