MTR

PDB:4CCZ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source:synthetic gene
SGC Clone Accession:
Tag:N-terminal, TEV protease cleavable hexahistidine tag
Host:

Construct

Prelude:
Sequence:

MHHHHHHSSGVDLGTENLYFQ*SMKTLRDEINAILQKRIMVLDGGMGTMIQREKLNEEHFRGQEFKDHARPLKGNNDILSITQPDVI
YQIHKEYLLAGADIIETNTFSSTSIAQADYGLEHLAYRMNMCSAGVARKAAEEVTLQTGIKRFVAGALGPTNKTLSVSPSVERPDYR
NITFDELVEAYQEQAKGLLDGGVDILLIETIFDTANAKAALFALQNLFEEKYAPRPIFISGTIVDKSGRTLSGQTGEGFVISVSHGE
PLCIGLNCALGAAEMRPFIEIIGKCTTAYVLCYPNAGLPNTFGDYDETPSMMAKHLKDFAMDGLVNIVGGCCGSTPDHIREIAEAVK
NCKPRVPPATAFEGHMLLSGLEPFRIGPYTNFVNIGERCNVAGSRKFAKLIMAGNYEEALCVAKVQVEMGAQVLDVNMDDGMLDGPS
AMTRFCNLIASEPDIAKVPLCIDSSNFAVIEAGLKCCQGKCIVNSISLKEGEDDFLEKARKIKKYGAAMVVMAFDEEGQATETDTKI
RVCTRAYHLLVKKLGFNPNDIIFDPNILTIGTGMEEHNLYAINFIHATKVIKETLPGARISGGLSNLSFSFRGMEAIREAMHGVFLY
HAIKSGMDMGIVNAGNLPVYDDIHKELLQLCEDLIWNKDPEATEKLLRYAQTQGTGGMHHHHHHSSGVDLGTENLYFQ*SM is th
e His6 tag folowed by TEV protease recognition site *.

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:

Purification

Buffers
Procedure
Cell Lysis Cell pellets were dissolved in approximately 50ml lysis buffer and broken by passing through a high pressure homogenizer at 15,000 psi for 4 cycles. The cell debris was pelleted at 35,000 x g and the supernatant used for further purification. Lysis Buffer: 50 mM Hepes pH 7.5, 500 mM NaCl, 5% Glycerol, 20 mM Imidazole pH 7.5, 0.5 mM TCEP, 1 tablet per 50 ml protease inhibitor cocktail EDTA-free (Roche) Step 1 Ni-NTA affinityThe clarified supernatant

was collected and incubated with 1.5 mL of Ni-NTA in cold room under rotation for 1h30min. The resin was then washed in a glass column with Binding Buffer (2x15 ml) and Wash Buffer (2x15 ml). The protein was eluted with 5x1 ml of Elution Buffer. All steps were done in cold room. Binding Buffer: 50 mM Hepes pH 7.5, 500 mM NaCl, 5% Glycerol, 20 mM Imidazole pH 7.5, 0.5 mM TCEP Wash Buffer: 50 mM Hepes pH 7.5, 500 mM NaCl, 5% Glycerol, 40 mM Imidazole pH 7.5, 0.5 mM TCEP Elution Buffer: 50 mM Hepes pH 7.5, 500 mM NaCl, 5% Glycerol, 250 mM Imidazole pH 7.5, 0.5 mM TCEP Step 2 Superdex 200 16/60 Gel FiltrationEluted fractions were concentrated to 5ml and loaded onto a Superdex 200 16/60 column (pre-equilibrated in GF Buffer) at 1.0 ml/min. 1.75 ml fractions were collected. GF Buffer: 10 mM Hepes pH 7.5, 500 mM NaCl, 0.5 mM TCEP, 5% Glycerol. Step 3 His-tag removalThe N-terminal His6-tag was cleaved by incubating overnight with TEV (20°C). Cleaved protein was purified by batch binding on 1ml pre-equilibriated Ni-NTA beads. The column was then washed with 2x1ml GF buffer, 2x1ml Wash buffer. Step 4 HiTrap Q HP Ion Exchange Cleaved protein was concentrated to 5 mL, and diluted with Zero Salt Buffer to a final NaCl concentration of 50 mM. The diluted sample was loaded onto the Hitrap Q HP anionic exchange column that was pre equilibrated with Low Salt Buffer. Elution was performed with a linear NaCl gradient from 0% to 25% of High Salt Buffer in 150 ml. 1.75 mL fractions were collected. Zero Salt Buffer: 50 mM HEPES pH 7.5, 5% Glycerol. Low Salt Buffer: 50 mM HEPES pH 7.5, 5% Glycerol, 50 mM NaCl. High Salt Buffer: 50 mM HEPES pH 7.5, 5% Glycerol, 2 M NaCl.

Extraction

Buffers

Procedure

Expression strain BL21(DE3)-R3-pRARE2Transformation The construct DNA was transformed into competent cells of the expression strain by a standard heat shock procedure. Glycerol stock preparation One colony from the transformation was used to inoculate 1 ml of TB media containing 50 µg/ml kanamycin and 50 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day glycerol stocks were prepared from this overnight culture. Expression A glycerol stock was used to inoculate 100 ml of TB media containing 50 µg/ml kanamycin and 50 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day this starter culture was used to inoculate 9 L of TB media (7.5 ml starter culture used per 1L) containing 50 µg/ml kanamycin. When the OD600 reached approximately 0.8 the temperature was reduced to 18°C and after a further 1 hour the cells were induced by the addition of 0.1 mM IPTG. The expression was continued overnight. Cell harvest Cells were harvested by centrifugation at 6000 x g after which the supernatant was poured out and the cell pellet either placed in a -20°C freezer or used directly for purification.

Concentration: The purified protein was concentrated to 14.7 mg/ml using Millipore 30k mwco concentrators.

Ligand

MassSpec: Measured mass: 70447.5 Da Expected mass: 70446.3 Da

Crystallization: Crystals were grown by the sitting drop vapour diffusion method at 20°C. A sitting drop consisting of 75 nl protein and 75 nl well solution was equilibrated against well solution containing 50 mM HEPES pH 7.5, 5% Glycerol. Crystals were mounted in the presence of 25 % (v/v) ethylene glycol and flash-cooled in liquid nitrogen.

NMR Spectroscopy:

Data Collection: Resolution: 2.7 Å X-ray source: Diamond Light Source beamline IO2

Data Processing: