NT5C2A

PDB:2J2C

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC001595
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated THROMB protease cleavage site: mgsshhhhhhssglvpr*gs.
Host:

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsMSTSWSDRLQNAADMPANMDKHALKKYRREAYHRVFVNRSLAMEKIKCFGFDMDYTLAVYKSPEYESL
GFELTVERLVSIGYPQELLSFAYDSTFPTRGLVFDTLYGNLLKVDAYGNLLVCAHGFNFIRGPETREQYPNKFIQRDDTERFYILNT
LFNLPETYLLACLVDFFTNCPRYTSCETGFKDGDLFMSYRSMFQDVRDAVDWVHYKGSLKEKTVENLEKYVVKDGKLPLLLSRMKEV
GKVFLATNSDYKYTDKIMTYLFDFPHGPKPGSSHRPWQSYFDLILVDARKPLFFGEGTVLRQVDTKTGKLKIGTYTGPLQHGIVYSG
GSSDTICDLLGAKGKDILYIGDHIFGDILKSKKRQGWRTFLVIPELAQELHVWTDKSSLFEELQSLDIFLAELYKHLDSSSNERPDI
SSIQRRIKKVTHDMDMCYGMMGSLFRSGSRQTLFASQVMRYADLYAASFINLLYYPFSYLFRAAHVLMPHESTVEHTHVDINEMESP
LATRNRTSVDFKDTDYKRHQLTRSISEIKPPNL

Vector:p28A-LIC

Growth

Medium:
Antibiotics:
Procedure: Rosetta2(DE) cells from glycerol stocks were grown in 20 mL Terrific Broth media supplemented with 8 g/L glycerol, 100 microG/mL kanamycin and 34 microG/mL chloramphenicol at 30 degC overnight. The following morning, 20 mL of the over night culture inoculated 4x750 mL of Terrific Broth media supplemented with 50 microG/mL kanamycin, 8 g/L glycerolin glass. Cells were grown at 37 degC and 225 rpm until OD600 of 1 and were down-tempered to 18 degC. After 50 minutes, expression of target protein was induced by addition of IPTG to a concentration of 0.5 mM. Protein expression was allowed to continue over night at 18 degC.

Purification

Procedure

Columns: 1 mL His-Trap HP (Ni-charged; GE Healthcare). Superdex 200 HiLoad 16/60 (GE Healthcare).
The sample was purified on an ÄKTA-prime (GE Healthcare). Briefly, sample was loaded on the IMAC column, eluted in 1 ml fractions. The protein containing fractions were pooled and then loaded on the gel filtration column. Elution fractions were pooled based on SDS-PAGE analysis. Protein was estimated by SDS-PAGE analysis to be more than 95% pure. Fresh TCEP was added to the pooled samples so that the concentration of TCEP was 2 mM. Concentration was performed by use of Amicon Ultra 15 (Millipore) with 30 000 MW CO, at 15 °C in swing-out buckets for 15 minutes at 3000 g. Final concentration was 7.4 mg/ml.

Extraction

Procedure
Cells were harvested by centrifugation and pellets were resuspended in 50 mM HEPES pH 7.5, 500mM NaCl, 10mM imidazol and 10% glycerol supplemented with one tablet Complete EDTA-free protease inhibitor tablet per 50 ml. Following additon of 2000 U Benzonase, cells were disrupted by High Pressure Homogenization run twice at 10 000 PSI and samples were centrifuged for 20 minutes at 40000×g. The soluble fraction was filtered through 0.45 µm and subjected to further purification using an ÄKTA Prime system.
Concentration:
Ligand
MassSpec:
Crystallization: Crystallization was performed using the vapour diffusion method with sitting drops containing 0.1 µl of protein solution (7.4 mg/mL) and 0.1 µl well solution (1.8 M magnesium sulfate and 0.1 M Tris, pH 8.5). Crystals appeared after x days.
NMR Spectroscopy:
Data Collection:
Data Processing: Native data to 2.2 Å was collected at ESRF, beam-line ID29. The space group is I222 and the cell parameters are 91.5 128.0 130.4 90 90 90. The structure was solved with one polypeptide per asymmetric unit, by Molecular Replacement using the structure of Legionella pneumophila NT5C2 (pdb entry: 2bde) as template. The structure comprises residues 3-488 compared to the 561 residues full length protein. Refmac5.2 was used for refinement and Coot for model building. TLS refinement with 10 TLS groups was used in Refmac.