NUDT3

PDB:2FVV

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC007727
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*s(m).
Host:E. coli Bl21(DE3) (Novagen)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsMMKLKSNQTRTYDGDGYKKRAACLCFRSESEEEVLLVSSSRHPDRWIVPGGGMEPEEEPSVAAVR
EVCEEAGVKGTLGRLVGIFENQERKHRTYVYVLIVTEVLEDWEDSVNIGRKREWFKIEDAIKVLQYHKPVQASYFETLRQGYS

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 overnight. The following morning 20 mL of the overnight cultures were used to inoculate two TunAir flasks (Shelton Scientific) with 750 mL of phosphate buffered TB with 50 mg/l Kan. The culture was incubated at 37°C, until OD600 reached approximately 1. The temperature was lowered to 18°C and the cultures were induced with 0.5 mM IPTG. The expression proceeded overnight.

Purification

Procedure
Columns: 1 ml Hi-Trap Chelating (Ni-charged). (GE Healthcare). Superdex 75 HiLoad 16/60 (GE Healthcare).
The sample was purified automatically on an ÄKTA-Xpress (GE Healthcare). Briefly, sample was loaded on the IMAC column, eluted in a storage loop 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 10 000 MW CO. Centrifugation was performed at 15 deg in swing-out buckets for 15 minutes at 3000 g. Yield of purified protein per liter of culture was 5.9 mg.

Extraction

Procedure
Cells were harvested by centrifugation (WCW 28.7 g) and pellets were resuspended in 60 ml of lysis buffer (50 mM Na-Phosphate, 500 mM NaCl, 10% glycerol, 10 mM imidazole, 0.5 mM TCEP and 2 tablets Complete EDTA-free protease inhibitor (Roche Biosciences)) before freezing at -80 degrees Celsius. After thawing, 4 ?l of a 250 U/?l benzonase (Novagen) stock solution was added. Cells were then disrupted by high pressure homogenization with a high-pressure homogenizer (Stansted) (2 passes) prior to centrifugation for 30 min at 49000 g in a Sorvall SS-34 rotor. The soluble fraction was decanted and filtered through 0.45 µm.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained using the sitting drop method at 4°C. Drops were prepared using 100 nl of protein (11 mg/ml concentration) and 100 nl of the well solution (30% Peg 8000, 200 mM lithium sulphate) using a Phoenix crystallization robot (Art Robbins Instruments, Sunnyvale, CA, USA). 0.1 mm plates grew after three weeks. The structure was solved to 2.0 Å resolution in spacegroup P212121 by sulphur SAD using data collected with a Bruker Proteum in-house X-ray source.
NMR Spectroscopy:
Data Collection:
Data Processing: