NAT2

PDB:2PFR

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:4557783
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQ*G
Host:E.coli BL21 (DE3) codon plus RIL (Stratagene)

Construct

Prelude:
Sequence:

ggsgsDIEAYFERIGYKNSRNKLDLETLTDILEHQIRAVPFENLNMHCGQAMELGLEAIFDHIVRRNRGGWCLQVNQLLYWALTTIG
FQTTMLGGYFYIPPVNKYSTGMVHLLLQVTIDGRNYIVDAGSGSSSQMWQPLELISGKDQPQVPCIFCLTEERGIWYLDQIRREQYI
TNKEFLNSHLLPKKKHRKIYLFTLEPRTIEDFESMNTYLQTSPTSSFITTSFCSLQTPEGVYCLVGFILTYRKFNYKDNTDLVEFKT
LTEEEVEEVLKNIFKISLGRNLVPKPGDGSLTI

Vector:pEET28-MHL

Growth

Medium:TB
Antibiotics:
Procedure:NAT2 was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 μg/ml of kanamycin. Cell were grown at 37 degC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 15 degC

Purification

Procedure
The crude extract was cleared by centrifugation. The clarified lysate was loaded onto 10 ml Chelating Sepharose column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of washing buffer containing 20 mM Tris HCl, pH 8.0, 500 mM NaCl and 50 mM imidazole, 5% glycerol. The protein was eluted with elution buffer. The protein was dialyzed against 20 mM Tris HCl, pH 8.0, 250 mM NaCl, 5% glycerol in the presence of TEV protease. The dialyzed protein was passed through a 5 ml Ni HiTrap column and loaded on Source 30Q column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM Tris HCl, pH 8.5, and

eluted with linear gradient of NaCl up to 0.5 M concentration (20CV). Purification yield was 0.5 mg of the protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80 degC. For the purification the cell paste was thawed and resuspended in lysis buffer. The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:4 mg/ml
Ligand
MassSpec: Expected mass=33784.77 Da, measured mass =33756.7398 Da.
Crystallization: Purified NAT2 protein was complexed with CoA at 1:10 molar ratio of protein: CoA and crystallized using the sitting drop vapor diffusion method by mixing 1 microL of protein solution with 1 microL of the reservoir solution containing 2.5 M Ammonium sulfate, 0.1 M Tris HCl, pH 8.5.
NMR Spectroscopy:
Data Collection:
Data Processing: