SULT1B1

PDB:3CKL

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_055280
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPR*GS
Host:E. coli BL21(DE3) codon plus RIL (Stragagen)

Construct

Prelude:
Sequence:

gsMLSPKDILRKDLKLVHGYPMTCAFASNWEKIEQFHSRPDDIVIATYPKSGTTWVSEIIDMILNDGDIEKCKRGFITEKVPMLEMT
LPGLRTSGIEQLEKNPSPRIVKTHLPTDLLPKSFWENNCKMIYLARNAKDVSVSYYHFDLMNNLQPFPGTWEEYLEKFLTGKVAYGS
WFTHVKNWWKRKEEHPILFLYYEDMKENPKEEIKKIIRFLEKNLNDEILDRIIHHTSFEVMKDNPLVNYTHLPTTVMDHSKSPFMRK
GTAGDWKNYFTVAQNEK FDAIYETEMSKTALQFRTEI

Vector:pET28a-LIC

Growth

Medium:TB
Antibiotics:
Procedure:SULT1B1 was expressed in E. coli BL21(DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 µg/mL of kanamycin at 37ºC to an OD600 of 0.8. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.5 mM and incubated overnight at 15ºC.

Purification

Procedure
Column 1: 5 mL HiTrap Chelating column (Amersham Biosciences)
Column 2: Source 30Q column (10x10) (Amersham Biosciences)

The clarified lysate was loaded onto 5 mL HiTrap Chelating column (Amersham Biosciences), charged with Ni2+. The column was washed with wash buffer (10 CV), and the protein was eluted with elution buffer. The purified protein was dialyzed against buffer 20 mM Tris-HCl, pH 8.0, 150 mM NaCl and treated with thrombin (Sigma) overnight at 4ºC. The protein was further

purified to homogeneity by ion-exchange chromatography on Source 30Q column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM Tris-HCl, pH 8.0, and eluted with linear gradient of NaCl up to 500 mM concentration (30CV). Purification yield was 26 mg of the protein per 1L of culture.

Extraction

Procedure
Extraction procedure Cultures were centrifuged and the cell pellets were frozen in liquid nitrogen and stored at -80ºC. For purification the cell paste was thawed and resuspended in lysis buffer with protease inhibitor (0.1µM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.).
Concentration:36 mg/mL
Ligand
MassSpec: Expected: 35071.3
Measured: 35071.0
Crystallization: Purified SULT1B1 was pre-incubated with 3\'-phosphoadenosine 5\'-phosphate (PAP) and Resveratrol at 1:10:30 molar ratio of protein:PAP:resveratrol and crystallized using the sitting drop vapor diffusion method at 20 ºC by mixing 2 µL of the protein-compound mixture with 2 µL of the reservoir solution containing 0.1 M Bis-Tris, pH 6.0, 0.001M DTT, 0.1 M ammonium sulfate and 17% polyethylene glycol 3350.
NMR Spectroscopy:
Data Collection:
Data Processing: