SULT1B1

PDB:2Z5F

Revision

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Revised by:created
Revision Date:created
Entry Clone Accession:gi:4507305
Entry Clone Source:MGC
SGC Clone Accession:SULT1B1_01:; plate APC003:D9
Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (DE3) codon plus RIL (Stratagene)

Construct

Prelude:
Sequence:

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gsMLSPKDILRKDLKLVHGYPMTCAFASNWEKIEQFHSRPDDIVIA              TYPKSGTTWVSEIIDMILNDGDIEKCK
RGFITEKVPMLEMTLPGLRTSGIEQLEKNPS                       PRIVKTHLPTDLLPKSFWENNCKMIYLARNAKDVSV
SYYHFDLMNNLQPFPGTWEEYL                      EKFLTGKVAYGSWFTHVKNWWKRKEEHPILFLYYEDMKENPKEEI
KKIIRFLEKNLND                     EILDRIIHHTSFEVMKDNPLVNYTHLPTTVMDHSKSPFMRKGTAGDWKNYFTVA
QNEK                    FDAIYETEMSKTALQFRTEI
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Vector:p28a-LIC

Growth

Medium:
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 37°C 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 15°C.

Purification

Buffers
Procedure
Column 1: The clarified lysate was loaded onto 5 mL HiTrap Chelating column (Amersham Biosciences), charged with Ni2+. The column was washed with 20mM Tris-HCl buffer , pH 8.0, containing 500 mM NaCl and 50 mM imidazole (10 CV), and the protein was eluted with elution buffer (20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 250 mM imidazole).

Column 2: The purified protein was dialyzed against buffer 20 mM Tris-HCl, pH 8.0, 150 mM NaCl and treated with thrombin (Sigma) overnight at 4°C. 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

Buffers
Procedure
Cultures were centrifuged and the cell pellets were frozen in liquid nitrogen and stored at -80 °C. For purification the cell paste was thawed and resuspended in lysis buffer (phosphate buffer saline (PBS), pH 7.5, 0.5 M NaCl, 5% glycerol) with protease inhibitor (0.1 µM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.).
Concentration:36 mg/mL
Ligand
MassSpec:
Crystallization: Purified SULT1B1 was complexed with 3ï¿½Phosphoadenosine 5ï¿½-phosphate (PAP) at 1:5 molar ratio of protein:PAP and crystallized using the hanging drop method at 20 °C by mixing 2 µL of the protein solution with 2 µL of the reservoir solution containing 0.1 M BisTris, pH 6.5, 0.2 M ammonium sulfate and 16-20% polyethylene glycol 4000.
NMR Spectroscopy:
Data Collection:
Data Processing: