SULT1C1

PDB:2ETG

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi:45935387
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (DE3) codon plus RIL (Stratagen).

Construct

Prelude:
Sequence:

gsLTSDLGKQIKLKEVEGTLLQPATVDNWSQIQSFEAKPDDLLICTYPKAGTTWIQEIVDMIEQNGDVEKCQRAIIQHRHPFIEWAR
PPQPSGVEKAKAMPSPRILKTHLSTQLLPPSFWENNCKFLYVARNAKDCMVSYYHFQRMNHMLPDPGTWEEYFETFINGKVVWGSWF
DHVKGWWEMKDRHQILFLFYEDIKRDPKHEIRKVMQFMGKKVDETVLDKIVQETSFEKMKENPMTNRSTVSKSILDQSISSFMRKGT
VGDWKNHFTVAQNERFDEIYRRKMEGTSINFsMEL

Vector:p28a-LIC

Growth

Procedure:SULT1C1 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

Procedure
The clarified lysate was loaded onto 5 mL HiTrap Chelating column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM Tris-HCl buffer, pH 8.5, containing 500 mM NaCl and 50 mM imidazole, and the protein was eluted with elution buffer (20 mM Tris-HCl, pH 8.5, 500 mM NaCl, 250 mM imidazole). The protein was loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM Tris-HCl buffer, pH 8.5, and 150 mM NaCl, at flow rate 4 mL/min. Thrombin (Sigma) was added to combined fractions containing SULT1C1 and incubated 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 8.5, and eluted with linear gradient of NaCl up to 500 mM concentration (30CV). Purification yield was 72 mg of the protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation at 6,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80°C. For the purification the cell paste was thawed and resuspended in lysis buffer (30 mM Tris-HCl, pH 8.5, 0.5 M NaCl, 5 mM imidazol, 2 mM &betha;-mercaptoethanol, 5% glycerol) with protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:34 mg/mL
Ligand
Crystallization: Purified SULT1C1 was complexed with 3'Phosphoadenosine 5'-phosphate (PAP) (Sigma) 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 K2HPO4 and 12-16% polyethylene glycol 3350.