SULT4A1

PDB:1ZD1

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi:7657633
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 (Stratagene)

Construct

Prelude:
Sequence:

gsMAESEAETPSTPGEFESKYFEFHGVRLPPFCRGKMEEIANFPVRPSDVWIVTYPKSGTSLLQEVVYLVSQGADPDEIGLMNIDEQ
LPVLEYPQPGLDIIKELTSPRLIKSHLPYRFLPSDLHNGDSKVIYMARNPKDLVVSYYQFHRSLRTMSYRGTFQEFCRRFMNDKLGY
GSWFEHVQEFWEHRMDSNVLFLKYEDMHRDLVTMVEQLARFLGVSCDKAQLEALTEHCHQLVDQCCNAEALPVGRGRVGLWKDIFTV
SMNEKFDLVYKQKMGKCDLTFDFYL

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure:SULT4A1 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 ºC 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 ºC.

Purification

Procedure

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 (phosphate buffer saline (PBS), pH 7.4, 0.5 M NaCl, 5 mM imidazol, 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:

Ligand

MassSpec:

Crystallization:SULT4A1 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 20% polyethylene glycol 4000, 0.2 M ammonium tartrate

NMR Spectroscopy:

Data Collection:

Data Processing: