SAT

PDB:2FXF

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:4506789
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:

Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure:SAT 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 37oC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 15oC.

Purification

Procedure

Extraction

Procedure
Cells were harvested by centrifugation at 7,000 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 (1X phosphate-buffered-saline, 0.25 M NaCl, 5 mM imidazole, 2 mM ß-mercaptoethanol, 5% glycerol) with protease inhibitor (0.1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000

psi.
Concentration:
Ligand
MassSpec:
Crystallization: Purified SAT was complexed with acetylcoenzyme A (AcCoA, Sigma) at 1:10 molar ratio of protein:AcCoA and crystallized using the hanging drop vapor diffusion method at 20 °C by mixing 1.5 µl of the protein solution with 1.5 µl of the reservoir solution containing 18% PEG4000, 0.2 M Ca Acetate, 0.1 M HEPES, pH 7.5.
NMR Spectroscopy:
Data Collection:
Data Processing: