

HS3ST1

PDB:1ZRH

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:GI:52426776

Entry Clone Source:MGC

SGC Clone Accession:

Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHSSGLVPR*GS

Host:E.coli BL21 (DE3) codon plus RIL (Stratagene)

Construct

Prelude:

Sequence:

```
gsTLQDDVRDGVAPNGSAQQLPQTIIIGVRKGGTRALLEMLSLHPDVAAAENEVHFFDWEEHYSHGLGWYLSQMPFSWPHQLTVEKT  
PAYFTSPKVPERVYSMNPSIRLLLLLRDPSERVLSDYTQVFYNHMQKHKPYPSIEEFLVRDGRNLVDYKALNRSLYHVHMQNWLRF  
PLRHHIIVDGDRLIRDPFPEIQKVERFLKLSPOINASNFYFNKTKGFYCLRDSGRDRCLHESKGRAHPQVDPKLLNKLHEYFHEPNK  
KFFELVGRTFDWH
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Vector:p24a-LIC

Growth

Medium:

Antibiotics:

Procedure:HS3ST1 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 1 mM, and incubated overnight at 15°C.

Purification

Procedure

The crude extract was cleared by centrifugation and passing through 20-mL DE52 column equilibrated in 20 mM HEPES, pH 7.4, containing 500 mM NaCl. 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 HEPES, pH 7.4, containing 500 mM NaCl and 50 mM imidazole, 5% glycerol, and the protein was eluted with linear gradient of imidazole up to 250 mM (40CV). The protein was dialyzed against 20 mM HEPES, pH 7.4, 150 mM NaCl, 5% glycerol and treated with thrombin (Sigma) overnight.

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 purification, the cell paste was thawed and resuspended in lysis buffer (50 mM HEPES, pH 7.4, 0.5 M NaCl, 5 mM imidazol, 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 HS3ST1 was complexed with 3'-Phosphoadenosine 5'-phosphate (PAP, Sigma) at 1:10 molar ratio of protein: PAP and crystallized using the hanging drop vapor diffusion method by mixing 2 μ l of protein solution with 2 μ l of the reservoir solution containing 2.0-2.4 M Sodium Formate, 0.1 M Sodium Acetate, pH 4.6, 5% glycerol.

NMR Spectroscopy:

Data Collection:

Data Processing: