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

Construct

Prelude:
Sequence:

gsTLQDDVRDGVAPNGSAQQLPQTIIIGVRKGGTRALLEMLSLHPDVAAAENEVHFFDWEEHYSHGLGWYLSQMPFSWPHQLTVEKT
PAYFTSPKVPERVYSMNPSIRLLLILRDPSERVLSDYTQVFYNHMQKHKPYPSIEEFLVRDGRLNVDYKALNRSLYHVHMQNWLRFF
PLRHIHIVDGDRLIRDPFPEIQKVERFLKLSPQINASNFYFNKTKGFYCLRDSGRDRCLHESKGRAHPQVDPKLLNKLHEYFHEPNK
KFFELVGRTFDWH

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 37oC 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 15oC.

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 3Â□-Phosphoadenosine 5Â□-phosphate (PAP, Sigma) at 1:10 molar ratio of protein: PAP and crystallized using the hanging drop vapor diffusion method by mixing 2 ?l of protein solution with 2 ?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: