HSA9761

PDB:1ZQ9

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:56786140
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:

mgsshhhhhhssglvprgsNTGIGQHILKNPLIINSIIDKAALRPTDVVLEVGPGTGNMTVKLLEKAKKVVACELDPRLVAELHKRV
QGTPVASKLQVLVGDVLKTDLPFFDTCVANLPYQISSPFVFKLLLHRPFFRCAILMFQREFALRLVAKPGDKLYCRLSINTQLLARV
DHLMKVGKNNFRPPPKVESSVVRIEPKNPPPPINFQEWDGLVRITFVRKNKTLSAAFKSSAVQQLLEKNYRIHCSVHNIIIPEDFSI
ADKIQQILTSTGFSDKRARSMDIDDFIRLLHGFNAEGIHFS

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure:HSA9761 was expressed in E.coli BL21 (DE3) codon plus RIL in M9 minimal medium 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 1 mM, in the presence of 50 mg/L of SeMet (Bioshop) and incubated overnight at 15 ºC.

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 elution buffer (20 mM HEPES, pH 7.4, 500 mM NaCl, 250 mM imidazole, 5% glycerol). The protein was dialyzed against 20 mM HEPES, pH 7.4, 500 mM NaCl, 5% glycerol and further purified to homogeneity by ion-

exchange chromatography on Source 30S column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM HEPES pH 7.4, and eluted with linear gradient of NaCl up to 0.5 M concentration (20CV). Purification yield was 20 mg of the protein per 1L of culture.

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, 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:46 mg/mL
Ligand
MassSpec: Expected MW is 33879.5 Da. Measured MW is 33936.287 Da.
Crystallization: Purified HSA9761 was complexed with S-adenosyl-L-methionine (SAM, Sigma) at 1:10 molar ratio of protein: SAM and crystallized using the hanging drop vapor diffusion method by mixing 2 µL of protein solution with 2 µL of the reservoir solution containing 20% PEG 8000, 0.2 M Ammonium Sulfate, 0.1 M HEPES, pH 7.5, 8% glycerol.
NMR Spectroscopy:
Data Collection:
Data Processing: