EHMT1

PDB:2IGQ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:40217808
Entry Clone Source:
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (DE3) codon plus RIL (Stratagene).

Construct

Prelude:
Sequence:

gsNSQVWSALQMSKALQDSAPDRPSPVERIVSRDIARGYERIPIPCVNAVDSEPCPSNYKYVSQNCVTSPMNIDRNITHLQYCVCID
DCSSSNCMCGQLSMRCWYDKDGRLLPEFNMAEPPLIFECNHACSCWRNCRNRVVQNGLRARLQLYRTRDMGWGVRSLQDIPPGTFVC
EYVGELISDSEADVREEDSYLFDLDNKDGEVYCIDARFYGNVSRFINHHCEPNLVPVRVFMAHQDLRFPRIAFFSTRLIEAGEQLGF
DYGERFWDIKGKLFSCRCGSPKCRHS

Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure:EHMT1 was expressed in E.coli BL21 (DE3) codon plus RIL in TB medium in the presence of 50 µg/ml of kanamycin. Cell were grown at 37oC to an OD600 of 0.8 and 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 Tris, pH 8.0, containing 500 mM NaCl and 5% glycerol. The lysate was loaded onto 5 ml HiTrap column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM Tris pH 8.0, containing 250 mM NaCl and 50 mM imidazole, 5% glycerol, and the protein was eluted with elution buffer (20 mM Tris pH 8.0, 250 mM NaCl, 250 mM imidazole, 5% glycerol). The protein was dialyzed against 20 mM Tris, pH 8.0, 250 mM NaCl, 5% glycerol, 5mM ß-mercaptoethanol in the presence of thrombin (Sigma). The protein

was further purified to homogeneity by ion-exchange chromatography on Source 30Q column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM Tris-HCl, pH 8.0, and eluted with linear gradient of NaCl up to 500 mM concentration (20CV). Purification yield was 5.4 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 the purification the cell paste was thawed and resuspended in lysis buffer (50mM Tris pH 8.0, 0.25 M NaCl, 2 mM ß-mercaptoethanol, 5% glycerol, 0.1% Igepal) with protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:23 mg/ml
Ligand
MassSpec: Expected MW is 32935.24 Da, measured mass is 32893.84 Da.
Crystallization: Purified EHMT1 was crystallized in presence of S-adenosyl-L-homocysteine (SAH, Sigma) using hanging drop vapor diffusion method drop at 20 °C by mixing 1.5 µl of the protein solution with 1.5 µl of the reservoir solution containing 10% Isopropanol, 20% PEG 4000, 0.1M Hepes pH 7.5.
NMR Spectroscopy:
Data Collection:
Data Processing: