

EHMT1

PDB:2RFI

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:

Entry Clone Source:MGC

SGC Clone Accession:GI:40217808

Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHSSGLVPRGS

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

Construct

Prelude:

Sequence:

gsNSQWWSALQMSKALQDSAPDRPSPVERIVSRDIARGYERIPIPCVAVDSEPCPSNYKYVSQNCVTSPMNIDRNITHLQYCVCID
DCSSSNMCGQLSMRCWYDKDGRLLPEFNMAEPLIFECNHACSCWRNCRNRVVQNGLRARLQLYRTRDMGVGVRSQDIPPGTFVC
EYVGELESDSEADVREEDSYLFDLNDKGEVYCIDARFYGNVSRFINHHCEPNLVPVRVFMHQDLRFPRIAFFSTRLIEAGEQLGF
DYGERFWDIKGKLFSCRCGSPKCRHS

Vector:pET28a-LIC

Growth

Medium:TB

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 β-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.

Enzymatic treatment: Thrombin

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 β -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: EHMT1-AdoHcy-H3K9me complex (protein/AdoHcy/peptide molar ratio of 1:5:10) was crystallized in 16% PEG 4,000, 10 % isopropanol, 0.1 M HEPES (pH 7.5) using hanging drop vapor diffusion method drop at 20 °C by mixing 1.5 μ l of the protein solution with 1.5 μ l of the reservoir solution.

NMR Spectroscopy:

Data Collection:

Data Processing: