

EHMT2

PDB:2H8K

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:GI:18375637

Entry Clone Source:MGC

SGC Clone Accession:

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

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

Construct

Prelude:

Sequence:

gsNRAIRTEKIIICRDVARGYENVPIPCVNGVDGEPCPCPEDYKYISENCETSTMNIDRNITHLQHCTCVDDCSSNCLGQLSIRCWYD
KGDRLLQEFNKIEPPLIFECNQACSCWRNCKNRVQSGIKVRLQLYRTAKMGWGVRALQTIPQGTFICEYVGELISDAEADVREDDSYLFDDNDNKGDEVYCIDARYYGNISRFINHLCDPNIIPVRVFMLHQDLRFPRIAFFSSRDIRTGEELGFDYGDRTWDIKSKYFTCQCG
SEKCKHSAEAIALEQSRLARLD

Vector:p28a-LIC

Growth

Medium:

Antibiotics:

Procedure:EHMT2 was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin. Cells were grown at 37oC to OD600 of 1.5 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 loaded onto 5 ml HiTrap Chelating column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM Tris-HCl buffer, pH 8.0, containing 250 mM NaCl, 50 mM imidazole and 5% glycerol, and the protein was eluted with elution buffer (20 mM Tris-HCl, pH 8.0, 250 mM NaCl, 250 mM imidazole, 5% glycerol). The protein was loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM Tris-HCl buffer, pH 8.0, and 150 mM NaCl, at flow rate 4 ml/min. Thrombin (Sigma) was added to combined fractions containing EHMT2 and incubated

overnight at 4°C. 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 3 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 (phosphate-buffered saline, pH 7.4, 0.25 M NaCl, 5 mM imidazol, 2 mM β -mercaptoethanol, 5% glycerol) with protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.

Concentration: 12 mg/ml

Ligand

MassSpec: expected MW = 32564.8 Da, measured MW = 32563.41 Da.

Crystallization: Purified EHMT2 was complexed with S-adenosyl-L-homocysteine (SAH) (Sigma) at 1:5 molar ratio of protein:SAH and crystallized using the hanging drop vapor diffusion method at 20 °C by mixing 1 μ l of the protein solution with 1 μ l of the reservoir solution containing 20% PEG 3350, 0.2M NaF, 0.1 M Bis-Tris propane, pH 7.5, 5% ethylene glycol.

NMR Spectroscopy:

Data Collection:

Data Processing: