EHMT1

PDB:4H4H

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:40217808
Entry Clone Source:MGC
SGC Clone Accession:EHMT1:APC019-E11:C18667
Tag:N-terminal: His-tag with integrated thrombin protease site:MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (DE3) codon plus RIL (Stratagene).

Construct

Prelude:
Sequence:

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gsNSQVWSALQMSKALQDSAPDRPSPVERIVSRDIARGYERIPIPCVNAVDSEPCPSNYKYVSQNCVTSPMNIDRNITHLQYCVCID
DCSSSNCMCGQLSMRCWYDKDGRLLPEFNMAEPPLIFECNHACSCWRNCRNRVVQNGLRARLQLYRTRDMGWGVRSLQDIPPGTFVC
EYVGELISDSEADVREEDSYLFDLDNKDGEVYCIDARFYGNVSRFINHHCEPNLVPVRVFMAHQDLRFPRIAFFSTRLIEAGEQLGF
DaGERFWDIKGKLFSCRCGSPKCRHS
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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 weregrown 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 passingthrough 20-ml DE52 column equilibrated in 20 mM Tris, pH 8.0, containing 500 mMNaCl and 5% glycerol. The lysate was loaded onto 5 ml HiTrap column (AmershamBiosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM TrispH 8.0, containing 250 mM NaCl and 50 mM imidazole, 5% glycerol, and the proteinwas 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 wasfurther purified to homogeneity by ion-exchange chromatography on Source 30Q column(10x10) (Amersham Biosciences), equilibrated with buffer 20 mM Tris-HCl, pH 8.0, andeluted with linear gradient of NaCl up to 500 mM concentration (20CV). Purificationyield was 5.4 mg of the protein per 1L of culture.

Enzymatic treatment: Thrombin

Extraction

Procedure
Cells were harvested by centrifugation at7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80˚C. For thepurification the cell paste was thawed and resuspended in lysis buffer (50mM Tris pH8.0, 0.25 M NaCl, 2 mM β-mercaptoethanol, 5% glycerol, 0.1% Igepal) with proteaseinhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed bypassing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:23 mg/ml
Ligand
MassSpec: Expected MW is 32801.8 Da, measured mass is 32893.84Da.
Crystallization: Purified EHMT1 was crystallized in presence of S-adenosyl-L-homocysteine (SAH, Sigma) and H3K9 Nε-allyl peptide using hanging drop vapordiffusion method drop at 20 °C by mixing 1.5 µl of the protein solution with 1.5 µl of thereservoir solution containing 10% Isopropanol, 20% PEG 4,000, 0.1M HEPES, pH 7.5.
NMR Spectroscopy:
Data Collection:
Data Processing: