DNMT3A

PDB:3LLR

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:12751473
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQG
Host:*E.coli* BL21 (DE3) codon plus RIL (Stratagene).

Construct

Prelude:
Sequence:

gGDDEPEYEDGRGFGIGELVWGKLRGFSWWPGRIVSWWMTGRSRAAEGTRWVMWFGDGKFSVVCVEKLMPLSSFCSAFHQATYNKQP
MYRKAIYEVLQVASSRAGKLFPVCHDSDESDTAKAVEVQNKPMIEWALGGFQPSGPKGLEPPEE

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure: The PWWP domain of DNMT3B was expressed in E.coli BL21 (DE3) codon plus in Terrific Broth medium in the presence of 50 µg/ml of kanamycin. Cell were grown at 37 degC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM and incubated overnight at 15 degC.

Purification

Procedure
The lysate was loaded onto 5 ml HiTrap column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of wash buffer, and the protein was eluted with elution buffer. The protein was then loaded on to a Superdex200 (60X60, Amersham Biosciences) column equilibrated in 20 mM Tris-HCl, pH 8.0 buffer containing 150 mM NaCl. TEV protease was added to combined fractions containing DNMT3A. 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 2.4 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 degC. For the purification the cell paste was thawed and resuspended in lysis buffer with protease inhibitor (1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:5.8 mg/ml.
Ligand
MassSpec: Expected MW is 16940.08 Da, measured mass is 16940.79 Da.
Crystallization: Purified PWWP domain of DNMT3A was crystallized using sitting drop vapor diffusion method at 20 degC by mixing 1.5 µl of the protein solution (5.8 mg/ml) with 1.5 µl of the reservoir solution containing 28% PEG 3,350, 0.1 M ammonium sulfate, 0.1 M BisTris, pH 6.0.
NMR Spectroscopy:
Data Collection:
Data Processing: