

DNMT3B

PDB:3QKJ

Revision

Revision Type:created

Revised by:created

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Entry Clone Accession:GI:5901940

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:

gEADSGDGDSSEYQDGKEFGIGDLVGKIKGFSWPAMVSWKATSKRQAMSGMRWVQWFGDGKFSEVSADKLVALGLFSQHFNLAT
FNKLVSYRKAMYHALEKARVRAKTFPSSPGDSLEDQLKPMLEAHGGFKPTGIEGLKPNNNTQP

Vector:pET28-MHL

Growth

Medium:Terrific Broth

Antibiotics:50 µg/ml of kanamycin

Procedure:The PWP 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 °C to an OD₆₀₀ of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM and incubated overnight at 15 °C.

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 HEPES, pH 7.4 buffer containing 250 mM NaCl. TEV protease was added to combined fractions containing DNMT3B. The cut and uncut protein of DNMT3B were separated on a Ni column. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30S column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM PIPES, pH 6.5, and eluted with linear gradient of NaCl up to 500 mM concentration (20CV). Purification yield was 8 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 degrees Celsius. 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:45.5 mg/ml

Ligand

MassSpec:Expected MW is 16733.83 Da

Measured MW is 16734.24 Da

Crystallization:Purified PWWP domain of DNMT3B was crystallized using sitting drop vapor diffusion method at 20 °C by mixing 1 μ l of the protein solution (22 mg/ml) with 1 μ l of the reservoir solution containing 27% PEG 3350, 0.2 M LiSO₄, 0.1 M BisTris, pH 6.0.

NMR Spectroscopy:

Data Collection:

Data Processing: