# DNMT3B

**PDB:**3FLG

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**NP_008823
**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:**

gEADSGDGDSSEYQDGKEFGIGDLVWGKIKGFSWWPAMVVSWKATSKRQAMSGMRWVQWFGDGKFSEVSADKLVALGLFSQHFNLAT
FNKLVSYRKAMYHALEKARVRAGKTFPSSPGDSLEDQLKPMLEWAHGGFKPTGIEGLKPNNTQP

**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 20 mM HEPES, pH 7.4, containing 500 mM NaCl and 50 mM imidazole, 5% glycerol, and the protein was eluted with elution buffer (20 mM HEPES pH 7.4, 500 mM NaCl, 250 mM imidazole, 5% glycerol). 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 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:**45.5 mg/ml.
**Ligand**
**MassSpec:**Expected MW is 16733.83 Da, measured mass is 16734.24 Da.
**Crystallization:**Purified PWWP domain of DNMT3B was crystallized using sitting drop vapor diffusion method at 20 °C by mixing 1 µl of the protein solution (22 mg/ml) with 1 µl of the reservoir solution containing 30% PEG 2,000 MME, 0.2 M KBr. Crystals were frozen in liquid nitrogen using glycerol as cryoprotectant.
**NMR Spectroscopy:**
**Data Collection:**
**Data Processing:**