METTL1

PDB:3CKK

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_005362
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQ*G
Host:*E.coli* BL21 (DE3) codon plus RIL (Stratagen).

Construct

Prelude:
Sequence:

gDHTLRYPVKPEEMDWSELYPEFFAPLTQNQSHDDPKDKKEKRAQAQVEFADIGCGYGGLLVELSPLFPDTLILGLEIRVKVSDYVQ
DRIRALRAAPAGGFQNIACLRSNAMKHLPNFFYKGQLTKMFFLFPDPHFKRTKHKWRIISPTLLAEYAYVLRVGGLVYTITDVLELH
DWMCTHFEEHPLFERVPLEDLSEDPVVGHLGTSTEEGKKVLRNGGKNFPAIFRRIQDPVLQ

Vector:pET28a-LIC

Growth

Medium:TB
Antibiotics:
Procedure:METTL1 protein 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 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
Column 1: 5 ml HiTrap column (Amersham Biosciences)
Column 2: Source 30S column (10x10) (Amersham Biosciences)

The crude extract was cleared by centrifugation. 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 fractions containing METTL1 protein was pooled and dialyzed against 20 mM HEPES buffer, pH 7.4, and 250 mM NaCl, in the presence of TEV, overnight at 4 degC. 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 7 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 with protease inhibitor (1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:28.5 mg/ml
Ligand
MassSpec: The expected mass for METTL1 is 26997.03 Da, measured mass is 26997.00 Da.
Crystallization: Purified METTL1 protein was complexed with S-adenosyl-L-methionine (SAM) (Sigma) at 1:10 molar ratio of protein:SAM and crystallized using sitting drop vapor diffusion method at 20°C by mixing 1 microL of the protein solution with 1 microL of the reservoir solution containing 2.0 M ammonium sulfate, 2% PEG400, 0.1 M Na-HEPES, pH 7.5.
NMR Spectroscopy:
Data Collection:
Data Processing: