METTL21A

PDB:4LEC

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:188528686
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQ*G
Host:E.coli BL21 (DE3) pRARE-V2R.

Construct

Prelude:
Sequence:

gETTEFGLQKFHKPLATFSFANHTIQIRQDWRHLGVAAVVWDAAIVLSTYLEMGAVELRGRSAVELGAGTGLVGIVAALLGAHVTIT
DRKVALEFLKSNVQANLPPHIQTKTVVKELTWGQNLGSFSPGEFDLILGADIIYLEETFTDLLQTLEHLCSNHSVILLACRIRYERD
NNFLAMLERQFTVRKVHYDPEKDVHIYEAQKRNQKEDL

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:METTL21A was expressed in E.coli BL21 (DE3) pRARE-V2R in Terrific Broth (TB) medium in the presence of 50 µg/ml of kanamycin. Cell were grown at 37oC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 15oC.

Purification

Procedure
The lysate was loaded onto 5 ml HiTrap column (GE Healthcare), charged with Ni2+. The column was washed with 10 CV of 20 mM Tris-HCl pH 8.0, containing 250 mM NaCl, 50 mM imidazole, 5% glycerol, and the protein was eluted with elution buffer (20 mM Tris-HCl pH 8.0, 250 mM NaCl, 250 mM imidazole, 5% glycerol). The protein was loaded on Superdex200 column (26x60) (GE Healthcare), equilibrated with 20 mM Tris-HCl buffer, pH 8.0, and 150 mM NaCl. The fractions containing METTL21A were pooled and TEV was added to remove His-tag. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30Q column (10x10) (GE Healthcare), equilibrated with buffer 20 mM Tris-HCl, pH 8.0, and

eluted with linear gradient of NaCl up to 500 mM concentration (20 CV). Purification yield was 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˚C. For the purification the cell paste was thawed and resuspended in lysis buffer (1X PBS, 250 mM NaCl, 2 mM β-mercaptoethanol, 5% glycerol, 0.1% CHAPS) with protease inhibitor (0.1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:31 mg/ml - Enzymatic treatment: TEV.
Ligand
MassSpec: Expected MW is 23853.3Da, measured mass is 23870.3074 Da.
Crystallization: Purified METTL21A (10 mg/ml) was complexed with S-adenosyl-homocysteine (SAH, Sigma) at 1:5 molar ratio of protein:SAH and crystallized using the sitting drop vapor diffusion method at 20 °C by mixing 1 µl of the protein solution with 1 µl of the reservoir solution containing 15% PEG3350, 0.1 M succinate acid, pH 7.0. Crystal was frozen in liquid nitrogen using 15% glycerol as cryoprotectant.
NMR Spectroscopy:
Data Collection:
Data Processing: