METTL21D

PDB:4LG1

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:98986323
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:

gSSLEDPLRSFVRVLEKRDGTVLRLQQYSSGGVGCVVWDAAIVLSKYLETPEFSGDGAHALSRRSVLELGSGTGAVGLMAATLGADV
VVTDLEELQDLLKMNINMNKHLVTGSVQAKVLKWGEEIEGFPSPPDFILMADCIYYEESLEPLLKTLKDISGFETCIICCYEQRTMG
KNPEIEKKYFELLQLDFDFEKIPLEKHDEEYRSEDIHIIYIRKKKSKFPS

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:METTL21D 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 METTL21D 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 6.5 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:27 mg/ml - Enzymatic treatment: TEV.
Ligand
MassSpec: Expected MW is 25202.9 Da, measured mass is 25203.2033 Da.
Crystallization: Purified METTL21D was complexed with S-adenosyl-L-methionine (SAM, 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 (10 mg/mL) with 1 µl of the reservoir solution containing 30% PEG4000, 0.2M LiSO4, 0.1M TrisHCl pH8.5. Crystal was frozen in liquid nitrogen using 20% ethylene glycol as cryoprotectant.
NMR Spectroscopy:
Data Collection:
Data Processing: