METTL3-METTL14

PDB:5TEY

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:METTL3:BC007449; METTL14:BC001650
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQ*G
Host: Sf9

Construct

Prelude:
Sequence:

METTL3: MSDTWSSIQAHKKQLDSLRERLQRRRKQDSGHLDLRNPEAALSPTFRSDSPVPTAPTSGGPKPSTASAVPELATDPELE
KKLLHHLSDLALTLPTDAVSICLAISTPDAPATQDGVESLLQKFAAQELIEVKRGLLQDDAHPTLVTYADHSKLSAMMGAVAEKKGP
GEVAGTVTGQKRRAEQDSTTVAAFASSLVSGLNSSASEPAKEPAKKSRKHAASDVDLEIESLLNQQSTKEQQSKKVSQEILELLNTT
TAKEQSIVEKFRSRGRAQVQEFCDYGTKEECMKASDADRPCRKLHFRRIINKHTDESLGDCSFLNTCFHMDTCKYVHYEIDACMDSE
APGSKDHTPSQELALTQSVGGDSSADRLFPPQWICCDIRYLDVSILGKFAVVMADPPWDIHMELPYGTLTDDEMRRLNIPVLQDDGF
LFLWVTGRAMELGRECLNLWGYERVDEIIWVKTNQLQRIIRTGRTGHWLNHGKEHCLVGVKGNPQGFNQGLDCDVIVAEVRSTSHKP
DEIYGMIERLSPGTRKIELFGRPHNVQPNWITLGNQLDGIHLLDPDVVARFKQKYPDGIISKPKNL - METTL14:MDSRLQEIRE
RQKLRRQLLAQQLGAESADSIGAVLNSKDEQREIAETRETCRASYDTSAPNAKRKYLDEGETDEDKMEEYKDELEMQQDEENLPYEE
EIYKDSSTFLKGTQSLNPHNDYCQHFVDTGHRPQNFIRDVGLADRFEEYPKLRELIRLKDELIAKSNTPPMYLQADIEAFDIRELTP
KFDVILLEPPLEEYYRETGITANEKCWTWDDIMKLEIDEIAAPRSFIFLWCGSGEGLDLGRVCLRKWGYRRCEDICWIKTNKNNPGK
TKTLDPKAVFQRTKEHCLMGIKGTVKRSTDGDFIHANVDIDLIITEEPEIGNIEKPVEIFHIIEHFCLGRRRLHLFGRDSTIRPGWL
TVGPTLTNSNYNAETYASYFSAPNSYLTGCTEEIERLRPKS

Vector:pFBOH-MHL

Growth

Medium:
Antibiotics:
Procedure: Sf9 cells were infected with virus and incubated at 27°C for 48-72 hours until cell viability drops to 70-80%.

Extraction

Buffers
Procedure
Cells were harvested by centrifugation at 4,000 rpm at 4°C for 15 minutes. After removing the medium, cells were washed with cold 1X PBS. PBS was removed after centrifugation and the pellet was resuspended in suspension buffer (20 mM Tris, pH 8.0, 250 mM NaCl, 5% glycerol, 2 mM β-mercaptoethanol, 0.6 % NP-40, 1 X protease inhibitor cocktail (Roche), 5 mM imidazole The cell pellets were frozen in liquid nitrogen and stored at -80°C.

Purification

Buffers
Procedure
For purification the cell paste was thawed and 3000 U of benzonase (Novagen) were added. Cells were lyzed by brief sonication. The clarified lysate was loaded onto a 2 mL TALON column (Clonetech). The column was washed with 50 column volumes of 20 mM Tris-HCl buffer, pH 8.0, containing 250 mM NaCl, 5% cglycerol and 5 mM imidazole, and the protein was eluted with elution buffer (20 mM Tris-HCl, pH 8.0, 250 mM NaCl, 5% glycerol, 250 mM imidazole). The eluted protein was loaded on a Superdex200 column (GE Healthcare), equilibrated with 20 mM HEPES buffer, pH 7.4, and 250 mM NaCl. Pooled fractions containing METTL3/METLTTL14 were further purified by another round of gel filtration after auto proteolysis.

Concentration:12.6 mg/ml
Ligand
MassSpec: Measured mass are 25413.84 Da and 33914.49 Da.
Crystallization: Purified METTL3/METTL14 (12 mg/mL) was complexed with S-adenosyl-L-methionine (SAM, Sigma) at 1:10 molar ratio of protein:SAM and crystallized using hanging drop vapor diffusion method at 20 °C by mixing 2 µl of the protein solution with 2 µl of the reservoir solution containing 20% PEG4000, 0.2 M MgCl2, 0.1 M Tris, pH 8.5.
NMR Spectroscopy:
Data Collection:
Data Processing: