PRMT6

PDB:5E8R

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:227908866
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQ*G
Host: Sf9

Construct

Prelude:
Sequence:

gMSQPKKRKLESGGGGEGGEGTEEEDGAEREAALERPRRTKRERDQLYYECYSDVSVHEEMIADRVRTDAYRLGILRNWAALRGKTV
LDVGAGTGILSIFCAQAGARRVYAVEASAIWQQAREVVRFNGLEDRVHVLPGPVETVELPEQVDAIVSEWMGYGLLHESMLSSVLHA
RTKWLKEGGLLLPASAELFIAPISDQMLEWRLGFWSQVKQHYGVDMSCLEGFATRCLMGHSEIVVQGLSGEDVLARPQRFAQLELSR
AGLEQELEAGVGGRFRCSCYGSAPMHGFAIWFQVTFPGGESEKPLVLSTSPFHPATHWKQALLYLNEPVQVEQDTDVSGEITLLPSR
DNPRRLRVLLRYKVGDQEEKTKDFAMED

Vector:pFBOH-MHL

Growth

Medium:
Antibiotics:
Procedure:PRMT6 was expressed in Sf9 cells

Purification

Procedure
For purification the cell paste was thawed and resuspended in lysis buffer containing 20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 5 mM imidazol, 2 mM β-mercaptoethanol, 5% glycerol, 0.6% NP-40, protease inhibitor cocktail (Roche), 3000 U of benzonase (Novagen). 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 500 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 Tris-HCl buffer, pH 8.0, and 150 mM NaCl. Pooled fractions containing HRMT1L6 were subjected to TEV treatment

to remove His-tag. The protein was further purified to homogeneity by ion-exchange chromatography.

Extraction

Procedure
Cells were harvested by centrifugation at 5,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80˚C.
Concentration:19.6 mg/ml - Enzymatic treatment: TEV
Ligand
MassSpec:expected mass is 42022.6 Da, measured mass is 42132.48 Da.
Crystallization: Purified HRMT1L6 (5.6 mg/ml) was mixed with S-adenosyl-L-homocysteine (SAH, Sigma) and MS023 at 1:5 molar ratio of protein:compound 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 10% PEG 3350, 0.2 M MgCl2, 0.1 M sodium cacodylate, pH 5.6. The reservoir solution containing 10% ethylene glycol was used as cryo protectant.
NMR Spectroscopy:
Data Collection:
Data Processing: