PRMT6

PDB:4HC4

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:227908866
Entry Clone Source:MGC
SGC Clone Accession:HRMT1L6:PBC001-A12:C213642
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:HRMT1L6 was expressed in Sf9 cells
Antibiotics:
Procedure:

Purification

Procedure
For purification the cell paste was thawed and resuspended inlysis buffer containing 20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 5 mM imidazol, 2 mMß-mercaptoethanol, 5% glycerol, 0.6% NP-40, protease inhibitor cocktail (Roche), 3000U of benzonase (Novagen). Cells were lyzed by brief sonication. The clarified lysatewas loaded onto a 2 mL TALON column (Clonetech). The column was washed with50 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 mMTris-HCl, pH 8.0, 250 mM NaCl, 5% glycerol, 250 mM imidazole). The eluted proteinwas 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 weresubjected to TEV treatment

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

Enzymatic treatment: TEV

Extraction

Procedure
Cells were harvested by centrifugation at 5,000rpm. The cell pellets were frozen in liquid nitrogen and stored at -80°C.
Concentration:16 mg/ml
Ligand
MassSpec:expected mass is 42022.6 Da, measured mass is 42132.48Da.
Crystallization: Purified HRMT1L6 (4.9 mg/ml) was complexed with (SAH, Sigma) at1:5 molar ratio of protein:SAH and crystallized using the hanging drop vapor diffusionmethod at 20 °C by mixing 1 µl of the protein solution with 1 µl of the reservoir solutioncontaining 15% PEG3350, 0.1 M succinate acid, pH 7.0.
NMR Spectroscopy:
Data Collection:
Data Processing: