EZH2

PDB:4MI0

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:21361095
Entry Clone Source:MGC
SGC Clone Accession:
Tag:C-terminal non-cleavable His-tag.
Host:SF9

Construct

Prelude:
Sequence:

mYQPCDHPRQPCDSSCPCVIAQNFCEKFCQCSSECQNRFPGCRCKAQCNTKQCPCYLAVRECDPDLCLTCGAADHWDSKNVSCKNCS
IQRGSKKHLLLAPSDVAGWGIFIKDPVQKNEFISEYCGEIISQDEADRRGKVYDKYMCSFLFNLNNDFVVDATRKGNKIRFANHSVN
PNCYAKVMMVNGDHRIGIFAKRAIQTGEELFFDYRYSQADALKYVGIEREMEIPhhhhhh

Vector:pFBOHMHL

Growth

Medium:
Antibiotics:
Procedure:EZH2 was expressed in SF9 cells. The cells at density 4mln/ml were infected with 20ml of P3 viral stocks and shaken at 100 RPM at 27˚C for 48-72 hours until the viability drops to 70-80%.

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 ß-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% glycerol and 5 mM imidazole, and the protein was eluted with elution buffer (20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 5% glycerol, 250 mM imidazole). The eluted protein was further purified to homogeneity on a Superdex200 column (GE Healthcare), equilibrated with 20 mM Tris-HCl buffer, pH 8.0, and 500 mM NaCl.

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:12 mg/ml
Ligand
MassSpec:expected mass is 26797.4 Da, measured mass is 26839.98 Da.
Crystallization: Purified EZH2 (10 mg/ml) was mixed with SAM at 1:10 molar ratio of protein:compound and crystallized using sitting drop vapor diffusion method at 20 °C by mixing 1 µl of the protein solution with 1 µl of the reservoir solution containing 20 % PEG3350, 0.1 M HEPES, pH 7.5, 0.2 M Li SO4.
NMR Spectroscopy:
Data Collection:
Data Processing: