SUV39H2

PDB:2R3A

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:34147611
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated thrombin protease site:
MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (DE3) codon plus RIL (Stratagen).

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsPKDNNKTLKPAIAEYIVKKAKQRIALQRWQDELNRRKNHKGMIFVENTVDLEGPPSDFYYINEYKPAP
GISLVNEATFGCSCTDCFFQKCCPAEAGVLLAYNKNQQIKIPPGTPIYECNSRCQCGPDCPNRIVQKGTQYSLCIFRTSNGRGWGVK
TLVKIKRMSFVMEYVGEVITSEEAERRGQFYDNKGITYLFDLDYESDEFTVDAARYGNVSHFVNHSCDPNLQVFNVFIDNLDTRLPR
IALFSTRTINAGEELTFDYQMKGSGDISSDSIDHSPAKKRVRTVCKCGAVTCRGYLN

Vector:pET28a-LIC

Growth

Medium:TB
Antibiotics:
Procedure:SUV39H2 was expressed in *E.coli* BL21 (DE3) codon plus RIL in Terrific Broth (TB) medium in the presence of 50 µg/ml of kanamycin at 37 degC to an OD600 of 1.5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 15 degC.

Purification

Procedure
Column 1: 5 ml HiTrap Chelating column (Amersham Biosciences)
Column 2: Superdex200 column (26x60) (Amersham Biosciences)
Column 3: Source 30S column (10x10) (Amersham Biosciences).

The crude extract was cleared by centrifugation. The clarified lysate was loaded onto 5 ml HiTrap Chelating column (Amersham Biosciences), charged with Ni2+. The column was washed with 10

CV of 20 mM Tris-HCl buffer, pH 8.0, containing 250 mM NaCl and 50 mM imidazole, and the protein was eluted with elution buffer. The protein was loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM PIPES buffer, pH 6.5, and 250 mM NaCl, at flow rate 4 ml/min. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30S column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM PIPES, pH 6.5, and eluted with linear gradient of NaCl up to 500 mM concentration (20CV). Purification yield was 1.4 mg of the protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation at 12, 227 Xg. The cell pellets were frozen in liquid nitrogen and stored at -80˚C. For the purification, 11 g of the cell paste was thawed and resuspended in 110 ml lysis buffer with protease inhibitor (1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:4.55 mg/ml
Ligand
MassSpec:expected MW =35775.41 Da.
measured MW= 35775.8779 Da.
Crystallization: Purified SUV39H2 was complexed with S-adenosyl-L-methionine (SAM) (Sigma) at 1:10 molar ratio of protein:SAM and crystallized using the sitting drop vapor diffusion method at 20 °C by mixing 2 µl of the protein solution with 1 µl of the reservoir solution containing 20% PEG 10, 000, 0.1 M HEPES pH 7.5.
NMR Spectroscopy:
Data Collection:
Data Processing: