SETMAR

PDB:3F2K

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_006506
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQG
Host:*E.coli* BL21 (DE3) codon plus RIL (Stratagen).

Construct

Prelude:
Sequence:

gWVPHELTENQKNRRFEVSSSLILRNHNEPFLDRIVTCDEKWILYDNRRRSAQWLDQEEAPKHFPKPILHPKKVMVTIWWSAAGLIH
YSFLNPGETITSEKYAQEIDEMNQKLQRLQLALVNRKGPILLHDNARPHVAQPTLQKLNELGYEVLPHPPYSPDLLPTNYHVFKHLN
NFLQGKRFHNQQDAENAFQEFVESQSTDFYATGINQLISRWQKCVDCNGSYFD

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:SETMAR transposase domain was expressed in *E.coli* BL21 (DE3) codon plus RIL in M9 minimal medium in the presence of 50 μg/ml of kanamycin. Cell were grown at 37 degC to an OD600 of 0.8 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, in the presence of 50 mg/L of SeMet and incubated overnight at 15 degC.

Purification

Procedure
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 wash buffer, and the protein was eluted with elution buffer. The protein was loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM Tris-HCl buffer, pH 8.0, and 150 mM NaCl, at flow rate 4 ml/min. TEV protease was added to the pooled fractions containing SETMAR transposase domain and incubated at 4 degC overnight. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30Q column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM Tris-HCl, pH 8.0, and

eluted with linear gradient of NaCl up to 500 mM concentration (20CV). Purification yield was 8.8 mg of the protein per liter 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 degC. For purification, the cell paste was thawed and resuspended in 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:15.8 mg/ml.
Ligand
MassSpec:expected MW (SeMet derivative) = 26797.14 Da
measured MW= 26797.5134 Da.
Crystallization: Purified SETMAR transposase domain was 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 20% PEG 3,350, 0.2 M di-Na Tartrate.
NMR Spectroscopy:
Data Collection:
Data Processing: