SETMAR

PDB:3BO5

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:

gAEFKEKPEAPTEQLDVACGQENLPVGAWPPGAAPAPFQYTPDHVVGPGADIDPTQITFPGCICVKTPCLPGTCSCLRHGENYDDNS
CLRDIGSGGKYAEPVFECNVLCRCSDHCRNRVVQKGLQFHFQVFKTHKKGWGLRTLEFIPKGRFVCEYAGEVLGFSEVQRRIHLQTK
SDSNYIIAIREHVYNGQVMETFVDPTYIGNIGRFLNHSCEPNLLMIPVRIDSMVPKLALFAAKDIVPEEELSYDYSGRYLNLTVSAS
KERLDHGKLRKPCYCGAKSCTAFLPFDSS

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:SETMAR 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 30Q 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 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. 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 0.6 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 degC. 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:3.67 mg/ml
Ligand
MassSpec: Expected MW = 32258.73 Da
Measured MW= 32259.9936 Da.
Crystallization: Purified SETMAR was complexed with S-adenosyl-L-homocysteine (SAH) (Sigma) at 1:5 molar ratio of protein:SAH 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 25% PEG 3,350, 0.2 M ammonium acetate, 0.1 M Bis-Tris pH 5.5.
NMR Spectroscopy:
Data Collection:
Data Processing: