Target: EMG1

Entry clone source: MGC

Entry clone accession: GI:194328699

Construct (coding) sequence:
gSGGEQAQDWDALPPKRPRLGAGNKIGGRRLIVVLEGASLETVKVGKTYELLNCDKHKS
ILLKNGRDPGEARPDITHQSLLMLMDSPLNRAGLLQVYIHTQKNVLIEVNPQTRIPRTFDR
FCGLMVQLLHKLSVRAADGPQKLLKVIKNPVSDHFPVGCMKVGTSFSIPVVSDVRELVP
SSDPIVFVVGAFAHGKVSVEYTEKMVSISNYPLSAALTCAKLTTAFEEVWGVI

Vector: pET28-MHL

Tags and additions: N-terminal: 6XHis-tag with integrated TEV protease site:
MHHHHHHSSGRENLYFQ*G

Host: E.coli BL21 (DE3) codon plus RIL (Stratagen).

Growth medium, induction protocol: EMG1 protein was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 μg/ml of kanamycin. Cells were grown at 37oC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 15oC.

Extraction buffer, extraction method: Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80˚C. For purification, the cell paste was thawed and resuspended in lysis buffer buffer (50 mM HEPES, pH 7.4, 0.5 M NaCl, 5 mM imidazol, 2 mM β-mercaptoethanol, 5% glycerol) with protease inhibitor (1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.

Protein Purification: The crude extract was cleared by centrifugation. The lysate was loaded onto 5 ml HiTrap column (GE Healthcare), charged with Ni2+. The column was washed with 10 CV of 20 mM HEPES pH 7.4, containing 500 mM NaCl, 50 mM imidazole, 5% glycerol, and the protein was eluted with elution buffer (20 mM HEPES pH 7.5, 500 mM NaCl, 250 mM imidazole, 5% glycerol). The protein was loaded on Superdex200 column (26x60) (GE Healthcare), equilibrated with 20 mM PIPES, pH 6.5, 250 mM NaCl. The fractions containing EMG1 were pooled and TEV protease was added to remove His-tag. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30S column (10x10) (GE Healthcare), equilibrated with buffer containing 20 mM PIPES, pH 6.5, and eluted with linear gradient of NaCl up to 500 mM concentration (20 CV).

Stock Concentration: 10.8 mg/mL

Enzymatic treatment: TEV

Mass spec characterization: The expected mass for EMG1 is 25333.5 Da, measured mass is 25333.3678 Da.

Crystallization: Purified EMG1 protein (4.9 mg/mL) was complexed with S-adenosyl-L-homocysteine (SAH) (Sigma) at 1:5 molar ratio of protein:SAH and crystallized using sitting drop vapor diffusion method at 20 °C by mixing 1 ml of the protein solution with 1 ml of the reservoir solution containing 20% PEG3350, 0.2M di-NH4 Citrate.