MLH1

PDB:3RBN

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI: 4557757
Entry Clone Source:MGC
SGC Clone Accession:MLH1:JMC01L-G02:C205695
Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQG
Host:E.coli BL21 (DE3) V2R-pRARE

Construct

Prelude:
Sequence:

gSRKEMTAACTPRRRIINLTSVLSLQEEINEQGHEVLREMLHNHSFVGCVNPQWALAQHQTKLYLLNTTKLSEELFYQILIYDFANF
GVLRLSEPAPLFDLAMLALDSPESGWTEEDGPKEGLAEYIVEFLKKKAEMLADYFSLEIDEEGNLIGLPLLIDNYVPPLEGLPIFIL
RLATEVNWDEEKECFESLSKECAMFYSIRKQYISEESTLSGQQSEVPGSIPNSWKWTVEHIVYKALRSHILPPKHFTEDGNILQLAN
LPDLYK

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:MLH1 was expressed in E.coli BL21 (DE3) V2R-pRARE in M9 medium in the presence of 50 µg/ml of kanamycin. Cell were grown at 37°C to an OD600 of 1.5 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°C .

Purification

Procedure
The lysate was loaded onto 5 ml HiTrap column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM Tris-HCl, pH 8.0, containing 250 mM NaCl and 5% glycerol. The lysate was loaded onto 5 ml HiTrap column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM Tris-HCl pH 8.0, containing 250 mM NaCl and 50 mM imidazole, 5% glycerol, and the protein was eluted with elution buffer (20 mM Tris-HCl pH 8.0, 250 mM NaCl, 250 mM imidazole, 5% glycerol).The protein was then loaded on to a Superdex200 (26x60, Amersham Biosciences) column equilibrated in 20 mM Tris-HCl,

pH 8.0 buffer containing 150 mM NaCl. 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 23 mg of the protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80°C. For the purification the cell paste was thawed and resuspended in lysis buffer (50mM Tris pH 8.0, 0.25 M NaCl, 2 mM β-mercaptoethanol, 5% glycerol, 0.1% CHAPS) with protease inhibitor (1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:22 mg/ml
Ligand
MassSpec: Expected MW is 32996.2 Da, measured mass is 32998.3 Da.
Crystallization: Purified MLH1 was crystallized using sitting drop vapor diffusion method at 20 °C by mixing 1 µl of the protein solution (8 mg/ml) with 1 µl of the reservoir solution containing 22% PEG 4,000, 0.2 M NaoAc, 0.1 M Tris-HCl, pH 8.0.
NMR Spectroscopy:
Data Collection:
Data Processing: