MLH1

PDB:3NA3

Revision

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

Construct

Prelude:
Sequence:

gMSFVAGVIRRLDETVVNRIAAGEVIQRPANAIKEMIENCLDAKSTSIQVIVKEGGLKLIQIQDNGTGIRKEDLDIVCERFTTSKLQ
SFEDLASISTYGFRGEALASISHVAHVTITTKTADGKCAYRASYSDGKLKAPPKPCAGNQGTQITVEDLFYNIATRRKALKNPSEEY
GKILEVVGRYSVHNAGISFSVKKQGETVADVRTLPNASTVDNIRSIFGNAVSRELIEIGCEDKTLAFKMNGYISNANYSVKKCIFLL
FINHRLVESTSLRKAIETVYAAYLPKNTHPFLYLSLEISPQNVDVNVHPTKHEVHFLHEESILERVQQHIESKLLGSNSS

Vector:pET28-MHL

Growth

Medium: Terrific Broth medium in the presence of 50 µg/ml of kanamycin
Antibiotics:
Procedure:MLH1 was expressed in *E.coli* BL21 (DE3) V2R-pRARE in Terrific Broth medium in the presence of 50 µg/ml of kanamycin. Cell were grown at 37°C to an OD_{600} of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM and incubated overnight at 15°C.

Purification

Procedure

The lysate was loaded onto 5 mL HiTrap column (Amersham Biosciences), charged with Ni^{2+}. The column was washed with 10 CV of wash buffer and the protein was eluted with elution buffer. The protein was then loaded on to a Superdex200 (60X60, Amersham Biosciences) column equilibrated in 20 mM PIPES, pH 6.5 buffer containing 250 mM NaCl. TEV protease was added to combined fractions containing MLH1. 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 23 mg of the protein per 1L of culture.

Enzymatic Treatment: TEV cleavage

Extraction

Procedure

Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80oC. For the 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:16.6 mg/ml
Ligand
MassSpec: Expected MW is 37675.00Da, measured mass is 37676.8 Da.
Crystallization: Purified MLH1 was complexed with ADP and 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 20% PEG 4,000, 10% isopropanel, 0.1 M HEPES, pH 7.5.
NMR Spectroscopy:
Data Collection:
Data Processing: