PRDM9

PDB:4IJD

Revision

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Entry Clone Accession:GI:147905620
Entry Clone Source:MGC
SGC Clone Accession:JMC058-D11
Tag:N-terminal: His-tag with integrated TEV protease site: MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (DE3) codon plus RIL (Stratagene).

Construct

Prelude:
Sequence:

gSEPQDDDYLYCEMCQNFFIDSCAAHGPPTFVKDSAVDKGHPNRSALSLPPGLRIGPSGIPQAGLGVWNEASDLPLGLHFGPYEGRI
TEDEEAANNGYSWLITKGRNCYEYVDGKDKSWANWMRYVNCARDDEEQNLVAFQYHRQIFYRTCRVIRPGCELLVWYGDEYGQELGI
KWGSKWKKELMAGREPKPEIHPCPSCCLAFSSQKFLSQHVERNHSSQN

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:PRDM9 was expressed in E.coli BL21 (DE3) codon plus in M9 minimal medium in the presence of 50 µg/ml of kanamycin. Cell were grown at 37°C 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°C .

Purification

Procedure
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 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 loaded onto Superdex200 column (26x60) (GE Healthcare), 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 combined fractions containing PRDM9 and incubated overnight at 4°C . The protein was further purified to homogeneity by ion-exchange chromatography on Source 30Q column

(10x10) (GE Healthcare), 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 1 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 (1X PBS, 0.25 M NaCl, 3 mM ß-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:8.8 mg/ml - Enzymatic treatment: TEV
Ligand
MassSpec: Expected MW for SeMet labelled protein is 25372.2Da, measured mass is 25371.98 Da.
Crystallization: Purified PRDM9 (8 mg/ml) was crystallized using hanging drop vapor diffusion method at 20 °C by mixing 1.5 µl of the protein solution with 1.5 µl of the reservoir solution containing 23% PEG 3350, 0.2 M ammonium acetate, 0.1 M BisTris, pH 5.5.
NMR Spectroscopy:
Data Collection:
Data Processing: