PRDM4

PDB:3DB5

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_036538
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPRGS
Host:*E.coli* BL21 (DE3) codon plus RIL (Stratagene).

Construct

Prelude:
Sequence:

gsEHGPVTFVPDTPIESRARLSLPKQLVLRQSIVGAEVGVWTGETIPVRTCFGPLIGQQSHSMEVAEWTDKAVNHIWKIYHNGVLEF
CIITTDENECNWMMFVRKARNREEQNLVAYPHDGKIFFCTSQDIPPENELLFYYSRDYAQQIGVPE

Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure:PRDM4 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 degC 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 degC.

Purification

Procedure
Column 1: DE52 column.
Column 2: 5 ml HiTrap column (Amersham Biosciences)
Column 3: Superdex200 column (26x60) (Amersham Biosciences)
Column 4: Source 30Q column (10x10) (Amersham Biosciences)

The crude extract was cleared by centrifugation and passing through 20-ml DE52 column equilibrated in 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 wash buffer, and then 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. Thrombin (Sigma) was added to combined fractions containing PRDM2 and incubated overnight at 4 degC. 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 6.3 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 degC. For the purification the cell paste was thawed and resuspended in lysis buffer with protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:19.2 mg/ml.
Ligand
MassSpec: Expected MW for SeMet labelled protein is 17651.83 Da, measured mass is 17649.7881 Da.
Crystallization: Purified PRDM4 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 6.5. Crystal was frozen in liquid nitrogen using paratone-N as cryoprotectant.
NMR Spectroscopy:
Data Collection:
Data Processing: