PRMD11

PDB:3RAY

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:41349466
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQ*G
Host:*E.coli* BL21 (DE3) V2RpRARE

Construct

Prelude:
Sequence:

gDSSAMEVEPKKLKGKRDLIVPKSFQQVDFWFCESCQEYFVDECPNHGPPVFVSDTPVPVGIPDRAALTIPQGMEVVKDTSGESDVR
CVNEVIPKGHIFGPYEGQISTQDKSAGFFSWLIVDKNNRYKSIDGSDETKANWMRYVVISREEREQNLLAFQHSERIYFRACRDIRP
GEWLRVWYSEDYMKRLHSMSQETIHRNLARGEKRLQREKSEQVLDNPEDLRGPIHLSVLRQGK

Vector:pET28a-MHL

Growth

Medium: Terrific Broth (TB) medium
Antibiotics:50 µg/ml of kanamycin
Procedure:PRDM11 was expressed in *E.coli* BL21 (DE3) V2RpRARE in Terrific Broth (TB) 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 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. The fractions containing PRDM11 were pooled and TEV protease was added to the fractions and incubated at 4 degrees Celsius overnight. 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 10.2 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 degrees Celsius. For the purification the cell paste was thawed and resuspended in lysis buffer with protease inhibitor (0.1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:38.8 mg/ml
Ligand
MassSpec: Expected MW is 27434.9 Da
Measured MW is 27436.347 Da
Crystallization: Purified PRDM11 (10.1 mg/mL) was crystallized using hanging drop vapor diffusion method at 20 °C by mixing 1 µl of the protein solution with 1 µl of the reservoir solution containing 20 % PEG3350, 0.2 M KCl.
NMR Spectroscopy:
Data Collection:
Data Processing: