PRDM1

PDB:3DAL

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_001189
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:

mgsshhhhhhssglvprgsKMDMEDADMTLWTEAEFEEKCTYIVNDHPWDSGADGGTSVQAEASLPRNLLFKYATNSEEVIGVMSKE
YIPKGTRFGPLIGEIYTNDTVPKNANRKYFWRIYSRGELHHFIDGFNEEKSNWMRYVNPAHSPREQNLAACQNGMNIYFYTIKPIPA
NQELLVWYCRDFAERLHYPYPGELTMMNLTQ

Vector:pET28a-LIC

Growth

Medium:TB
Antibiotics:
Procedure:PRDM1 was expressed in *E.coli* BL21 (DE3) codon plus in TB medium in the presence of 50 µg/ml of kanamycin. Cell were grown at 37 degC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 15 degC.

Purification

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

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 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

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 26 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. The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:39 mg/ml.
Ligand
MassSpec: Expected MW for is 23724.59 Da, measured mass is 23593.8807 Da.
Crystallization: Purified PRDM1 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 2.0 M Na Formide, 0.1 M sodium acetate, pH 4.6.
NMR Spectroscopy:
Data Collection:
Data Processing: