PRDM12

PDB:3EP0

Revision

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Revised by:created
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Entry Clone Accession:NP_067632
Entry Clone Source:MGC
SGC Clone Accession:GI:15042949
Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPRGS
Host:*E.coli* BL21 (DE3) codon plus RIL (Stratagene).

Construct

Prelude:
Sequence:

gsKTAFTAEVLAQSFSGEVQKLSSLVLPAEVIIAQSSIPGEGLGIFSKTWIKAGTEMGPFTGRVIAPEHVDICKNNNLMWEVFNEDG
TVRYFIDASQEDHRSWMTYIKCARNEQEQNLEVVQIGTSIFYKAIEMIPPDQELLVWYGNSHNTFLGIPGVPGLEEDQKKNKHED

Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure:PRDM12 was expressed in *E.coli* BL21 (DE3) codon plus in Terrific Broth (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
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 14.5 mg of the protein per 1L of culture.

Enzymatic treatment: Thrombin.

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:39.8 mg/ml
Ligand
MassSpec: Expected MW is 19219.67 Da, measured mass is 19219.9691 Da.
Crystallization: Purified PRDM12 was crystallized using hanging drop vapor diffusion method at 20 °C by mixing 1 µl of the protein solution (5.0 mg/mL) with 1 µl of the reservoir solution containing 19% PEG 2,000 MME, 0.1 M KSCN. Crystal was frozen in liquid nitrogen using glycerol as cryoprotectant.
NMR Spectroscopy:
Data Collection:
Data Processing: