

PRDM10

PDB:3IHX

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:GI:41349458

Entry Clone Source:MGC

SGC Clone Accession:

Tag:N-terminal: His-tag with integrated TEV protease site: MGSSHHHHHHSSGLVPRGS

Host:*E.coli* BL21 (DE3) codon plus RIL (Stratagene).

Construct

Prelude:

Sequence:

gKHGPLHPINRPVLTRARASLPLVLYIDRFLGGVFSKRRIPKRTQFGPVEGPLVRGSELKDCYIHLKVSLDKGDRKERDLHEDLWF
ELSDETLCNWMFVRPAQNHLEQNLVAYQYGHVYYTTIKNVEPKQELKVWYAASYAEFVNQKIHD

Vector:pET28a-MHL

Growth

Medium:TB

Antibiotics:

Procedure:PRDM10 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 PIPES buffer, pH 6.5, and 250 mM NaCl, at flow rate 4 ml/min. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30S column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM PIPES, pH 6.5, and eluted with linear gradient of NaCl up to 500 mM concentration (20CV). Purification yield was 5 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 (1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.

Concentration: 8 mg/ml.

Ligand

MassSpec: Expected MW is 17926.64 Da.

Crystallization: Purified PRDM10 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: