

PRMT3

PDB:4HSG

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:

Entry Clone Source:GI:44771198

SGC Clone Accession:

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

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

Construct

Prelude:

Sequence:

DLQEDEDGVYFSSYGHYGIHEEMLKDKIRTESYRDFIYQNPFIKDKVVLVDVGC GTGILSMFAAKAGAKKVLGVDQSEILYQAMDII
RLNKLEDITILIKGKIEEVHLPVEKVDVIIEWMGYLLFESMLDSVLYAKNKYLAKGGSVYPDICTISLVAVSDVNKHADRIAFWD
DVYGFKMSCMKKAVIPEAVVEVLDPKTLISEPCGIKHIDCHTTISIDLEFSSDFTLKITRTSMCTAIAGYFDIYFEKNCHNRVVFST
GPQSTKTHWKQTVFLLEKPF SVKAGEALKGKVT VHKNKKDPRSLTVTLTLNNSTQTYGLQ

Vector:pET28a-LIC

Growth

Medium:

Antibiotics:

Procedure:The overexpression of the recombinant protein in E. coli BL21 (DE3)pRARE2-V2R was induced by addition of 1 mM isopropyl-1-thio-D-galactopyranoside (IPTG) and overnight incubation at 15 oC.

Purification

Procedure

After clarification of the crude extract by centrifugation (16,000 rpm for one hour using Aventi-J 20-XPI, Beckman Coulter), the lysate was loaded onto DE52 ion-exchange resin and passed through a Ni-NTA column. The column was washed and protein was eluted by addition of 20 mM Tris buffer, pH 7.5, 500 mM NaCl, 5% glycerol, containing 30 mM and 250 mM imidazole, respectively. Protein sample was purified further using 6mL Resource Q Anion exchange column (Code No:17-1179-01, GE Healthcare) and ÄKTA FPLC (GE Healthcare) where the column was pre-equilibrated with 10 mM Tris buffer, pH 7.6, followed by 10 mM Tris buffer, pH 7.6 with 1 M NaCl and then re-equilibrated with 10 mM Tris buffer, pH 7.6. The

diluted protein sample (final NaCl concentration of 50 mM) was loaded onto a 6 mL ResourceQ column. The contaminant proteins bind and pure PRMT3 would be in flow through with an approximate purity of 95%. After adjusting the NaCl final concentration to 150 mM, the protein was concentrated, flash-frozen and stored at -80 °C.

Extraction

Procedure

Harvested cells were re-suspended in 20 mM Tris buffer, pH 7.5, with 500mM NaCl, 5 mM imidazole and 5% glycerol. The cells were lysed chemically followed by sonication at a frequency of 8.5 with ten seconds on and ten seconds off (Sonicator 3000, Misoni).

Concentration: Purified protein was concentrated using 15 mL concentrators with an appropriate molecular weight cut-off (Amicon Ultra-15 10,000 MWCO, Millipore) to a final value of 2.2 mg/mL.

Ligand

MassSpec:

Crystallization: PRMT3 was incubated at 1.1 mg/mL overnight with 1-(1,2,3-benzothiadiazol-6-yl)-3-(2-oxo-2-phenylethyl)urea (compound 1) at 1:30 molar ratio (PRMT3: compound 1). Following incubation, protein was concentrated to 3 mg/mL and crystallized using the sitting drop diffusion method at 20°C by mixing 1 µL of the protein solution with 1 µL of the reservoir solution containing 25% PEG 3350, 0.2 M Lithium Sulfate, 0.1 M HEPES, pH 7.5, vapor diffusion hanging drop, temperature 18°C. Prior to freezing, 0.1 µL of 100 mM compound 1 was added directly to the drop. Crystals were soaked for 30 min in the same buffer with 10% glycerol.

NMR Spectroscopy:

Data Collection:

Data Processing: