CDY1

PDB:2FBM

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:4757966
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 (Stratagen).

Construct

Prelude:
Sequence:

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MGSSHHHHHHSSGLVPRGSSTYRDIVVKKEDGFTQIVLSTRSTEKNALNTEVIKEIVNALNSAAADDSKLVLFSAAGSVFCCGLDFG
YFVKHLRNNRNTASLEMVDTIKNFVNTFIQFKKPIVVSVNGPAIGLGASILPLCDLVWANEKAWFQTPYTTFGQSPDGCSSITFPKM
MGKASANEMLIAGRKLTAREACAKGLVSQVFLTGTFTQEVMIQIKELASYNPIVLEECKALVRCNIKLELEQANERECEVLRKIWSS
AQGIESMLKIPLLGYKAA
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Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure:CDY1 was expressed in E.coli BL21 (DE3) codon plus RIL in M9 minimal medium in the presence of 50 µg/mL of kanamycin. Cell were grown at 37oC to an OD600 of 0.8 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, in the presence of 50 mg/L of SeMet and incubated overnight at 15oC.

Purification

Procedure
The crude extract was cleared by centrifugation and passing through 20-mL DE52 column equilibrated in 20 mM Hepes, pH 7.5, containing 500 mM NaCl and 5% glycerol. The lysate was loaded onto 5 mL HiTrap column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM Hepes pH 7.5, containing 500 mM NaCl and 50 mM imidazole, 5% glycerol, and the protein was eluted with elution buffer (20 mM Hepes pH 7.5, 500 mM NaCl, 250 mM imidazole, 5% glycerol). The protein was dialyzed against 20 mM Hepes, pH 7.5,

250 mM NaCl, 5% glycerol, 5mM ß-mercaptoethanol. Purification yield was 5.8 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°C. For the purification the cell paste was thawed and resuspended in lysis buffer (50mM Hepes pH 7.5, 0.5 M NaCl, 2 mM ß-mercaptoethanol, 5% glycerol, 0.1% Igepal) with protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:
Ligand
MassSpec:
Crystallization: Purified CDY1 was crystallized using hanging drop vapor diffusion method drop at 20 °C by mixing 1.5 µl of the protein solution with 1.5 µl of the reservoir solution containing 2.0M Ammonium Sulfate, 0.2M K/Na Tartrate, 0.1M Bis-Tris pH 5.5.
NMR Spectroscopy:
Data Collection:
Data Processing: