CDYL2

PDB:4HAE

Revision

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Entry Clone Accession:gi229462825
Entry Clone Source: Open Biosystems
SGC Clone Accession:CDYL2:JMC01M-G09:C206444
Tag:N-terminal: His-tag with integrated TEV protease site:MHHHHHHSSGRENLYFQG
Host:E.coli BL21(DE3)-V2R-pRARE2

Construct

Prelude:
Sequence:

MHHHHHHSSGRENLYFQGASGDLYEVERIVDKRKNKKGKWEYLIRWKGYGSTEDTWEPEHHLLHCEEFIDEFNGLHMSKDK

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:CDYL2 chromo domain was expressed inE.coli BL21(DE3)-V2R-pRARE2 in Terrific Broth (TB) in the presence of 50 µg/ml ofkanamycin and 30 mg/mL chloramphenicol at at 37°C to an OD600 of 2.0, the temperatureof the medium was lowered to 16 degree and the culture was induced with 0.4 mM IPTG.The cells were allowed to grow overnight before harvesting.

Purification

Procedure
The lysate was centrifuged at 16,000 rpm for 45 minutes and the supernatants weremixed with 8 mL 50% flurry of Ni-NTA beads and incubated at 4 degree on rotaryshaker for one hour. The mixture was then centrifuged at 2000 rpm for 5 min and thesupernatant discarded. The beads were then washed with 50 mL washing buffer, andfinally 25 mL the elution buffer. The protein further purified by a Superdex-75 gelfiltration column. Fractions containing the protein were collected and concentrated withAmicon Ultra-15 centrifugal filter. The purity of the preparation is tested by SDS-PAGEto be greater than 95%.

Extraction

Procedure
Cells were harvested by centrifugation at 6,000rpm. The cell pellets were frozen in liquid nitrogen and stored at -80°C.
= Extraction buffers =
20 mM Tris-HCl, pH 7.8, 250mM NaCl
= Extraction procedure =
Frozen cells from 2L TB culture were thawed and resuspended in 200 mL extractionbuffer and 8 uL benzonase (Sigma Catalog # E1014, 250U/uL), and lysed usingsonicator.
Concentration:50 mg/ml
Ligand
MassSpec:expected MW=9816.8 Da, not measured
Crystallization: Crystallization was setup in sitting drops with Red Wings screens initially. Diffractingcrystals were from initial screen plate for Red Wings F07.

Crystal used for structure determination was grown in 25% P3350 0.2M MgCl2, 0.1MTris buffer at pH 8.5.Crystals grow to a mountable size within 1 week
NMR Spectroscopy:
Data Collection:
Data Processing: