CDY

PDB:2FW2

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:47271406
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:

```
ITYRDIVVKKEDGFTQIVLSTRSTEKNALNTEVIKEMVNALNSAAADDSKLVLFSAAGSVFCCGLDFGYFVRHLRNDRNTASLEMVD
TIKNFVNTFIQFKKPIVVSVNGPAIGLGASILPLCDLVWANEKAWFQTPYTTFGQSPDGCSSITFPKMMGKASANEMLIAGRKLTAR
EACAKGLVSQVFLTGTFTQEVMIQIKELASYNAIVLEECKALVRCNIKLELEQANERECEVLRKIWSSAQGIESMLKYVENKIDEF
```

Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure:CDY was expressed in E.coli BL21 (DE3) codon plus RIL in TB 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 and incubated overnight at 15oC.

Purification

Procedure

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 (50 mM Hepes, pH 7.5, 0.5 M NaCl, 5 mM imidazol, 2 mM ß-mercaptoethanol, 5% glycerol) 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 CDY was crystallized using sitting drop vapor diffusion method drop at 20 °C by mixing 1.0 µL of the protein solution with 1.0 µL of the reservoir solution containing 8% PEG 4000, 0.1M Na Acetate, 0.1M Na Acetate pH 4.6.

NMR Spectroscopy:

Data Collection:

Data Processing: