

# CDC34

**PDB:**2OB4

## Revision

**Revision Type:**created

**Revised by:**created

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**Entry Clone Accession:**ubc54.BC018143.MGC.AT17-G3.pCMV-SPORT6

**Entry Clone Source:**

**SGC Clone Accession:**ubc54.007.184; plate SDC021B02

**Tag:**MGSSHHHHHSSGLVPR\*GS

**Host:**BL21 (DE3)

## Construct

**Prelude:**

**Sequence:**

MGSSHHHHHSSGLVPR\*GSPSSQKALLLELKGQLQEEPVEGFRVTLVDEGDLYNWEVAIFGPPNTYYEGGYFKARLKFPIDPPYSPP  
AFRFLTKMWHPNIYETGDVCISILHPPVDDPQSGELPSERWNPTQNVRTILLSVISLLNEPNTFSPANVDASVMYRKWESKGKDRE  
YTDIIRKQVLGTVDAERDGVKVP

**Vector:**pET28a-LIC

## Growth

**Medium:**

**Antibiotics:**

**Procedure:**Protein was expressed in *E. coli* BL21 (DE3) grown in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin at 37°C to an OD600 of 7.5. Protein expression was then induced with isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.05 mM, and incubated overnight at 15°C. The culture was centrifuged and the cell pellets were collected and stored at -80°C.

## Purification

### Procedure

The cleared lysate was loaded onto a TALON metal-affinity resin column (BD Biosciences) at 4°C (1.5 ml settled gel volume per liter original cell culture). The column was washed with 10 ml wash buffer A, 10 ml wash buffer B and then with 30 ml wash buffer A, and the protein was eluted with 6 ml elution buffer. The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with GF buffer and concentrated by ultrafiltration to a final protein concentration of 42 mg/ml using Amicon Ultra centrifugal filter with 10kD cutoff.

## Extraction

### Procedure

Cell pellets were resuspended in Lysis buffer (25 ml per liter culture), lysed using Microfluidizer at 18000 psi, and cleared by centrifugation.

### Concentration:

#### Ligand

#### MassSpec:

**Crystallization:** Crystals were grown in hanging drops by mixing 2  $\mu$ l protein solution with 2  $\mu$ l well solution (28% PEG 4000, 0.1 M Tris-HCl, pH 8.5, 0.2 M MgCl<sub>2</sub>, 1 mM DTT and 7.5 mM glycyl-glycyl-glycine) at 21°C. For cryoprotection, the crystals were soaked in well solution supplemented with 25% ethylene glycol.

#### NMR Spectroscopy:

#### Data Collection:

#### Data Processing: