# DLC1

**PDB:**3KUQ

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**BC054511.1
**Entry Clone Source:**MGC cDNA library: AT77-G5
**SGC Clone Accession:**HPC077-B11
**Tag:**N-terminal tag: mhhhhhhssgrenlyfq*g
**Host:**BL21-V2R-pRARE2

## Construct

**Prelude:**DLC1:S1074-L1283
Tag not removed
**Sequence:**

mhhhhhhssgrenlyfqgSVFGVPLTVNVQRTGQPLPQSIQQAMRYLRNHCLDQVGLFRKSGVKSRIQALRQMNEGAIDCVNYEGQS
AYDVADMLKQYFRDLPEPLMTNKLSETFLQIYQYVPKDQRLQAIKAAIMLLPDENREVLQTLLYFLSDVTAAVKENQMTPTNLAVCL
APSLFHLNTLKRENSSPRVMQRKQSLGKPDQKDLNENLAATQGLAHMIAECKKL

**Vector:**pET28-MHL

## Growth

**Medium:**
**Antibiotics:**
**Procedure:**LEX Bubbling. The target protein was expressed in E. coli by inoculating 100 mL of overnight culture grown in Luria-Bertani medium into a 2 L of Terrific Broth medium in the presence of 50 mg/mL kanamycin and 25 mg/mL chloramphenicol at 37 degC. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 degC and the culutre was induced with 1 mM IPTG. The cells were allowed to grow overnight before harvested and flash frozen in liquid nitrogen and stored at -80 degC.

## Purification

**Procedure**
The lysate was centrifuged at 15,000 rpm for 45 minutes and the supernatants were mixed with 5 mL 50% flurry of Talon Cobalt beads and incubated at 4 degC on rotary shaker for one hour. The mixture was then centrifuged at 2300 rpm for 5 min and the supernant discarded. The beads were then washed with washing buffer containing 30 mM and 75 mM Imidazole, and finally the elution buffer. The flow-through was collected and further purifed by a Superdex-75 gel filtraton

column pre-equilibrated with gel filtration buffer. Fractions containing the protein were collected and concentrated with Amicon Ultra-15 centrifugal filter. The purity of the preparation is tested by SDS-PAGE to be greater than 95%.

## Extraction

**Procedure**
Frozen cells from 2L TB culture were thawed and resuspended in 150 mL extraction buffer with freshly added 0.5% CHAPS, and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 3 uL benzonase (Sigma Catalog # E1014, 250U/uL), and lysed using microfluidizer at 15,000 PSI.
**Concentration:**40 mg/mL
**Ligand**
**MassSpec:**native protein expected 26004, measured 26004
**Crystallization:**Crystal used for structure determination was grown in 30% P3350, 0.2 M NaCl 5% glycerol, 20 mM HEPES at pH 7.5 in hanging drop setup. 50% glycerol was used as cryoprotectant.
Crystals grow to a mountable size within 1 week
**NMR Spectroscopy:**
**Data Collection:**
**Data Processing:**