KLC1

PDB:3NF1

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:AU40-H2
Entry Clone Source:MGC library
SGC Clone Accession:HPC09H-C05
Tag:mhhhhhhssgrenlyfqg
Host:BL21-V2R-pRARE2

Construct

Prelude:KNS2:G205-G497
Tag not removed
Sequence:

```
mhhhhhhssgrenlyfqgGGYEIPARLRTLHNLVIQYASQGRYEVAVPLCKQALEDLEKTSGHDHPDVATMLNILALVYRDQNKYKD
AANLLNDALAIREKTLGKDHPAVAATLNNLAVLYGKRGKYKEAEPLCKRALEIREKVLGKDHPDVAKQLNNLALLCQNQGKYEEVEY
YYQRALEIYQTKLGPDDPNVAKTKNNLASCYLKQGKFKQAETLYKEILTRAHEREFGSVDDENKPIWMHAEEREECKGKQKDGTSFG
EYGGWYKACKVDSPTVTTTLKNLGALYRRQGKFEAAETLEEAAMRSRKQG
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Vector:pET28-MHL

Growth

Medium: Terrific Broth medium in the presence of 50 mg/mL kanamycin and 25 mg/mL chloramphenicol
Antibiotics:
Procedure:LEX Bubbling. The target protein was expressed in *E. coli* by inoculating 50 mL of overnight culture grown in Luria-Bertani medium into a 2L of Terrific Broth medium in the presence of 50 mg/mL kanamycin and 25 mg/mL chloramphenicol at 37 °C. When OD_{600} reached ~3.0, the temperature of the medium was lowered to 15 °C and the culture 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 °C.

Purification

Procedure
The lysate was centrifuged at 15,000 rpm for 60 minutes and the supernatants were mixed with 4 mL 50% flurry of Ni-NTA beads and incubated at 4 degree on rotary shaker for one hour. The mixture was then centrifuged at 2500 rpm for 5 min and the supernant discarded. The beads were

then washed with washing buffer, 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 4L 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 sonication.
Concentration:20.5 mg/mL
Ligand
MassSpec: Protein expected 35379; measured 35380.2
Crystallization: Crystallization was setup using sitting drops with Red Wings and SGC-I screens in 0.25 uL protein solution plus 0.4 uL well solution ratio. Diffracting crystals were found from initial screen plate for Red Winds E2. Crystal used for structure determination was grown in 2.0M Ammonium Formate, 0.1M HEPES buffer at pH 7.5, 5mM TCEP. Paratone was used as cryoprotectant. Crystals grow to a mountable size within one week.
NMR Spectroscopy:
Data Collection:
Data Processing: