ECT2

PDB:3L46

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:MGC cDNA library: BC112086:AT129-H4
Entry Clone Source:MGC
SGC Clone Accession:HPC09I-D08
Tag:mhhhhhhssgrenlyfq*g
Host:BL21-V2R-pRARE2

Construct

Prelude:ECT2:V237-E330
Tag not removed
Sequence:

```
mhhhhhhssgrenlyfqgVPPFQDCILSFLGFSDEEKTNMEEMTEMQGGKYLPLGDERCTHLVVEENIVKDLPFEPSKKLYVVKQEW
FWGSIQMDARAGETMYLYEKANTPE
```

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 degree. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 degree 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 degree.

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 degree 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 were 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:18.3 mg/mL
Ligand
MassSpec:native protein expected 13121.77 (uncut)
measured 13131.42
Crystallization: Crystal used for structure determination was grown in optimized SGC-I condition A3 (drop C1 in 24-well opt. plate).
Crystal used for structure determination was grown in 1.5M NaCitrate, 0.1 M Tris pH 7.5 in hanging drop setup in the presense of 1:100 (w/w) subtilisin (protease#4).
Rod-shaped crystals grow to a mountable size within one week.
No cryo used.

Diffracting crystals were also seen from Red Wings screen condition E1 in the presense of 1:100 chymotrypsin or 1:100 dispase.

NMR Spectroscopy:
Data Collection:
Data Processing: