RICS

PDB:3IUG

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source: Codon Devices Synthesized: SGC cDNA library: DNA 03-G3:RICS
SGC Clone Accession:HPC099-B11
Tag:N-terminal tag: mhhhhhhssgrenlyfq*g
Host:BL21-V2R-pRARE2

Construct

Prelude:RICS:E18-E228
Tag not removed
Sequence:

```
mhhhhhhssgrenlyfqgERVFGCDLGEHLLNSGFEVPQVLQSCTAFIERYGIVDGIYRLSGVASNIQRLRHEFDSEHVPDLTKEPY
VQDIHSVGSLCKLYFRELPNPLLTYQLYEKFSDAVSAATDEERLIKIHDVIQQLPPPHYRTLEFLMRHLSLLADYCSITNMHAKNLA
IVWAPNLLRSKQIESACFSGTAAFMEVRIQSVVVEFILNHVDVLFSGRISMAMQE
```

Vector:pET28-mhl (GI:134105571)

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 µg/mL kanamycin and 25 µg/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 0.5 mM IPTG. The cells were allowed to grow overnight before harvested and flash frozen in liquid nitrogen and stored at -80 degC.

Purification

Buffers
Binding buffer: 20 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 2mM BME, 5mM Imidazole
Washing buffer(W1/W2): 20 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 2mM BME, 30mM Imidazole/75mM Imidazole
Elution buffer: 20 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 2mM BME, 300mM

Imidazole
Gel filtration buffer: 20 mM HEPES pH 7.5, 500 mM NaCl, 1mM TCEP
Procedure
The lysate was centrigued at 15,000 rpm for 45 minutes and the supernatants were mixed with 5 mL 50% flurry of Talon 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-trough 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%.

TEV does not cut off the tag from the protein well.The protein is modified by BME added in the buffer.

Extraction

Buffers
Extraction buffer: 20 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 2mM BME, 5mM Imidazole
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 µL benzonase (Sigma Catalog # E1014, 250U/µL), and lysed using microfluidizer at 15,000 PSI.
Concentration:16.8 mg/mL
Ligand
MassSpec: Native expected 26266.0, measured 26267.3, 26343.2 (+75.9 BME), 26418.9 (+151.9 2xBME)
Crystallization: Crystallization was setup using in situ proteolysis method in sitting drops with Red Wings and SGC-I screens initially. Diffracting crystals were found from initial screen drops. Crystal used for structure determination was grown in 30% PEG5000MME, 0.2M (NH4)2SO4, 0.1M MES buffer pH 6.5, with 1:100 subtilisin (w/w) protease in sitting drop setup. No cryoprotectant used.
Crystals grow to a mountable size within 24 hours
NMR Spectroscopy:
Data Collection:
Data Processing: