THYN1

PDB:5J3E

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source:LIFESEQ3941133 open biosystems
SGC Clone Accession:PBC006-A10
Tag:N-terminal His6-tag, removed
Host:BL21(DE3)V2R-pRARE2

Construct

Prelude:
Sequence:

```
gMSRPRKRLAGTSGSDKGLSGKRTKTENSGEALAKVEDSNPQKTSATKNCLKNLSSHWLMKSEPESRLEKGVDVKFSIEDLKAQPKQ
TTCWDGVRNYQARNFLRAMKLGEEAFFYHSNCKEPGIAGLMKIVKEAYPDHTQFEKNNPHYDPSSKEDNPKWSMVDVQFVRMMKRFI
PLAELKSYHQAHKATGGPLKNMVLFTRQRLSIQPLTQEEFDFVLSLEEKEPS DNA sequence has been verified by
sequencing
```

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:LEX Bubbling - For native protein: The target protein was over-expressed in E. coli at 37degree C by inoculating 20 mL of overnight culture grown in Luria-Bertani medium into 2 L TerrificBroth medium in the presence of 40 ug/mL kanamycin and 25 ug/mL chloramphenicol. When the OD600 of the culture reached ~1.5, the temperature was lowered to 18 degree and the culture was induced with 0.25 mM final IPTG concentration. The cells were allowed to grow overnight before harvested by centrifugation (5,000 rpm Beckman JLA-8.1000rotor 15 min) and flash frozen in liquid nitrogen and stored at -80 degree.

Purification

Buffers
Washing Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 50 mM imidazole - Elution Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 250 mM imidazole - Dialysis Buffer: 20 mM Hepes pH 7.4, 300 mM NaCl, 2 mM TCEP
Procedure

The lysate was centrifuged at 15,000 rpm for 30 minutes. The supernant was supplemented with 4 mL Ni-NTA resin (50% Slurry) and incubated on a rotary drum for 1 hour at 4 degree, then loaded onto Bio-rad gravity column.The beads deposited in the open column was then washed with 40 mL lysis buffer followed by 20 mL washing buffer. Bound proteins were eluted using 20 mL elution buffer. The N-terminal His-tag was reomoved by overnight incubation with TEV protease (1:30 w/w) at 4degree during dialysis against the dialysis buffer. Uncut proteins and TEVprotease were removed by passing the solution through 3mL Ni-NTA beads,andthe target protein was further purified by gel filtration on a HighLoad 16/60 Superdex 75 column (GE Healthcare)preequilibrated with gel filtration buffer. Fractions containing the targetprotein were pooled and concentrated by centrifugal filters (Amicon mwco10kDa). The yields of the protein was about 9 mg per litre bacterialculture with >95% purity.

Extraction

Buffers
50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol
Procedure
2L native cell pellet was resuspended in a total volume of 200 ml extraction buffer with1mM PMSF/Benzamidine freshly added and the cells disrupted by sonication for 10 mins at 5" on 10" off duty cycle at 108W output power.
Concentration: Concentration used for crystallization : 12.0 mg/mL
Ligand
MassSpec:measured (after His-tag cleavage): 25754.7 Da.
Crystallization: Crystals used for structure determination were grown at 293K in 96well sitting drop Vapor Diffusion method by mixing 0.5 uL protein solution with 0.5 uL well solution containing 0.1M TRISODIUM CITRATE, 0.2M NaCl, 30% PEG3350, pH 5.6.The crystals were cryoprotected by 20-30% Glycerol before flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection:
Data Processing: