EIF3J

PDB:3BPJ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source:
SGC Clone Accession:HPC075-B04
Tag:mhhhhhhssgrenlyfq*g
Host:BL21-CodonPlus(DE3)-RIL

Construct

Prelude:
Sequence:

```
mhhhhhhssgrenlyfqgKIAEKIKEKERQQKKRQEEIKKRLEEPEEPKVLTPEEQLADKLRLKKLQEESDLELAKETFGVNNAVYG
IDAMNPSSRDDFTEFGKLLKDKITQYEKSLYYASFLEVLVRDVCISLEIDDLKKITNSLTVLCSEKQKQEKQSKAK
```

Vector:pET28-mhl

Growth

Medium: Terrific Broth
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 1.8 L of Terrific Broth medium in the presence of 50 µg/mL kanamycin and chloramphenicol at 37 °C. When OD600 was ~3.0, the temperature of the media was lowered to 15 °C and the culutre was induced with 1mM IPTG, and the cells were allowed to grow overnight before harvesting and flash frozen in liquid nitrogen and stored at -80 °C before use.

Purification

Procedure
The lysate was centrigued at 16,000 rpm for 60 minutes and the supernatant was passed through two open columns filled with DE52 and 5 mL 50% Ni-NTA beads at 4 °C. The Ni beads were then washed using washing buffer and the proteins eluted using 16 mL elution buffer. The elutant was loaded onto Superdex-75 gel filtration column. Eluted fractions were pooled and concentrated using amicon centrifugal filter (m.w. cut-off 10,000). The purity of the proteins was higher than 95% judged by SDS-PAGE.

Extraction

Procedure
Frozen cells were thawed and suspended in 150 mL the binding buffer and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 2 µL (Sigma Catalog # E1014, 250U/ µL) benzonase, and lysed using Microfluidizer (French press).
Concentration:15 mg/mL, in 20 mM HEPES pH 7.5 150 mM NaCl 1 mM TCEP buffer
Ligand
N/A**MassSpec:**Measured 19131.5, expected 19130.7
Crystallization:
NMR Spectroscopy:
Data Collection:
Data Processing: