

# EIF4A2

PDB:3BOR

## Revision

**Revision Type:**created

**Revised by:**created

**Revision Date:**created

**Entry Clone Accession:**NP\_001958.2

**Entry Clone Source:**

**SGC Clone Accession:**HPC075-H02

**Tag:**mhhhhhssgrenlyfq\*g

**Host:**BL21-CodonPlus(DE3)-RIL

## Construct

**Prelude:**

**Sequence:**

mhhhhhssgrenlyfqgGVIESNWNEIVDNFDDMNLKESLLRGIYAYGF EKPSAIQQRAIIPC IKGYDVIAQAQSGTGKTATFAIS  
ILQQLIEIFKETQALVLAPTRELAQQIQKVILALGDYMGATCHACIGGTNVRNEMQKLQAEAPHIVVGTPGRVFDMLNRRYLSPKWI  
KMFVLDEADEMLSRGFKDQIYEIFQKLNTSIQVVLLSATMPTDVLEVTKKFMRDPIRILVKKE

**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 culture 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 centrifuged 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  $\mu$ L (Sigma Catalog # E1014, 250U/ $\mu$ L) benzonase, and lysed using Microfluidizer (French press).

**Concentration:** 15 mg/mL, in 20 mM HEPES pH 8.0 150 mM NaCl 1 mM TCEP buffer

### **Ligand**

N/A **MassSpec:** Measured 26894.2, expected 26893.0

### **Crystallization:**

### **NMR Spectroscopy:**

### **Data Collection:**

### **Data Processing:**