

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:

mhhhhhssgrenlyfqgGVIESNWNEIVDNFDDMLKESLLRGIFYAYGFEKPSAIQQRAIIPCIKGVDVIAQAGSGTGKTATFAIS
ILQQLEIEFKETQALVLAPTRELAQIQKVILALGDYMGATCHACIGGTNVRNEMQKLQAEAPHIVVGTPGRVFDMLNRRYLSPKWI
KMFVLDDEADEMLSRGFKDQIYEIFQKLNTSIQVVLLSATMPTDVLEVTKKFMRDPIRILVKKE

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 μ L (Sigma Catalog # E1014, 250U/ μ 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/AMassSpec: Measured 26894.2, expected 26893.0

Crystallization:

NMR Spectroscopy:

Data Collection:

Data Processing: