EIF5A

PDB:3CPF

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC000751
Entry Clone Source:MGC AU5-C4
SGC Clone Accession:HPC076-B07
Tag:mhhhhhhssgrenlyfq*g
Host:BL21-CodonPlus(DE3)-RIL

Construct

Prelude:
Sequence:

gSATFPMQCSALRKNGFVVLKGRPCKIVEMSTSKTGKHGHAKVHLVGIDIFTGKKYEDICPSTHNMDVPNIKRNDFQLIGIQDGYLS
LLQDSGEVREDLRLPEGDLGKEIEQKYDCGEEILITVLSAMTEEAAVAIKA

Vector:pET28-mhl (GI:134105571)

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 25 µg/mL chloramphenicol at 37 °reeC. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 µC 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 °C.

Purification

Procedure
The lysate was centrigued at 15,000 rpm for 45 minutes and the supernatants were mixed with 5 mL 50% flurry of Ni-NTA beads and incubated at 4°reeC 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 were collected and digested with TEV protease. TEV protease was removed from the treated protein sample by adding 100 uL

50% flurry of Ni-NTA beads and the sample was purified with supderdex-75 gel filtration again. Fractions containing the protein were collected and concentrated with Amicon Ultra-15 centrifugal filter. The purify of the preparation is tested by SDS-PAGE to be around 99%.

Extraction

Procedure
Frozen cells from 1.8L TB culture were thawed and resuspended in 150 mL extraction buffer with freshly added 0.5% CHAPS and 2mM BME, 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:29.5 mg/mL
Ligand
N/A**MassSpec:**Native: 15149.67, expected 15149.41
Crystallization: Crystallization was setup using sitting drops with Red Wings and SGC-I screens initially. Small crystals were seen at condition SC08.
Optimization was done using hanging drop vaporization.
Crystal used for structure determination were grown in: 22.0% PEG3350, 0.2M (NH4)2SO4, 0.1 M NaCaco pH 5.5. Cryo used 20% PEG 3350 + 20% EG.

Last updated by ytong 20080410
NMR Spectroscopy:
Data Collection:
Data Processing: