Entry Clone Source: MGC

Entry Clone Accession: IMAGE:4081713

SGC Construct ID: AASDHPPTA-c003

GenBank GI number: gi|20357568

Vector: pCOEX-1

Tags and additions (His 6 + TEV cleavage site):
mgsshhhhhhssgrenlyfqgh

Host : *E.coli* BL21 DE3-R3

Construct coding sequence:
mgsshhhhhhssgrenlyfqghMEGVRW
AFSCGTWLPSRAEWLLAVRSIQPEEKERI
GQFVFARDAKAAMAGRLMIRKLVAEKLNI
PWNHIRLQRTAKGKPVLAKDSSNPYPNFN
FNISHQGDYAVLAAEPELQVGIDIMKTSF
PGRGSIPEFFHIMKRKFTNKEWETIRSFK
DEWTQLDMFYRNWALKESFIKAIGVGLGF
ELQRLEFDLSPLNLDIGQVYKETRLFLDG
EEEKEWAFEESKIDEHHFVAVALRKPDGS
RHQDVPSQDDSKPTQRQFTILNFNDLMSS
AVPMTPEDPSFWDCFCFTEEIPIRNGTKS

Growth medium, induction protocol: Medium: TB + 34 µg/ml Chloramp. Cells were grown in 1 liter TB in 2.5-L baffled flasks, which was inoculated with 10 ml overnight culture. The culture was grown at 37°C to OD=2.3, and transfered to 25°C. 1 mM IPTG was then added, and incubation continued for 4 hours. The cells were then collected by centrifugation and frozen at -80°C.

Extraction buffer, extraction method: Lysis buffer: 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP, Complete® protease inhibitors (1 tablet/50 ml).

Frozen cell pellets were thawed on ice over night and resuspended in a total volume of 40 ml lysis buffer, the cells were disrupted by high pressure (20 psi) followed by sonication.

Nucleic acids and cell debris were removed by adding 0.15% PEI from a 5% (w/v) stock, stirring for 15 minutes, then centrifugation for 20 minutes at 40,000xg. The supernatant was then further clarified by filtration (0.45 µm).

Column 1 : Ni-affinity, HisTrap, 1 ml (GE/Amersham)

Buffers: Lysis buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM Imidazole, 5% glycerol, 0.5 mM TCEP. **Wash buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM Imidazole, 5% glycerol, 0.5 mM TCEP. **Elution buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, 30 mM Imidazole, 0.5 mM TCEP.

Procedure: The cell extract was loaded on the column at 0.8 ml/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10 volumes of Lysis buffer, 10 volumes of wash buffer, and then eluted with elution buffer at 0.8 ml/min. The eluted peak of A_{280} was automatically collected.

Column 2 : Gel filtration, Hiload 16/60 Superdex 200 prep grade 120 ml (GE Healthcare)

Buffers : GF buffer: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP.

Procedure: The eluted fractions from the Ni-affinity Histrap columns were loaded on the gel filtration column in GF buffer at 1.0 ml/min. Eluted proteins were collected in 1 ml fractions.

Concentration : In vivaspin 6 ml 10 K. Concentration determined from the absorbance at 280 nm using the extinction coefficient and the nanodrop method.
Concentration : 19.6 mg/ml
Enzymatic treatment : no cleavage
Mass spectrometry: observed mass corresponds to predicted protein sequence
Crystallization: 2BYD: Crystals were obtained using the following conditions: Vapour diffusion method, sitting drop, 293K, 0.05 M H3Cit/Na3Cit, pH 5.7, 14% PEG 3350. **2C43:** Crystals were obtained from 2.0 M NaCl and 10% PEG6000 at 293 K.
Data Collection: 2BYD: Resolution: 2.0Å, **X-ray source:** Synchrotron SLS -X10, multiple wavelength. **2C43: Resolution:** 1.9Å, **X-ray source:** Rigaku FRE, single wavelength.