Entry Clone Source: MGC

Entry Clone Accession: IMAGE:5419511

SGC Construct ID: GMFGA-c008

GenBank GI number: gi|4758440

Vector: pNIC28-Bsa4. Details [PDF]; Sequence [FASTA] or [GenBank]

Coding DNA sequence:

```
CATATGCACCATCATCATCATCATTCTTC
TGGTGTAGATCTGGGTACCGAGAACCTGT
ACTTCCAATCCATGGTGTGCGAGGTAGAC
CCAGAGCTAACAGAAAAGCTGAGGAAATT
CCGCTTCCGAAAAGAGACAGACAATGCAG
CCATCATAATGAAGGTGGACAAAGACCGG
CAGATGGTGGTGCTGGAGGAAGAATTTCA
GAACATTTCCCCAGAGGAGCTCAAAATGG
AGTTGCCGGAGAGACAGCCCAGGTTCGTG
GTTTACAGCTACAAGTACGTGCATGACGA
TGGCCGAGTGTCCTACCCTTTGTGTTTCA
TCTTCTCCAGCCCTGTGGGCTGCAAGCCG
GAACAACAGATGATGTATGCAGGGAGTAA
AAACAGGCTGGTGCAGACAGCAGAGCTCA
CAAAGGTGTTCGAAATCCGCACCACTGAT
GACCTCACTGAGGCCTGGCTCCAAGAAAA
GTTGTCTTTCTGACAGTAAAGGTGGATAC
GGATCCGAA
```

Tag sequence: N-terminal His-tag with a TEV protease cleavage site (*):

```
mhhhhhhssgvdlgtenlyfq(*)sm
```

Tag removed: yes

Host: BL21(DE3)-R3-pRARE2

Expressed protein sequence (tag sequence in lowercase):

```
mhhhhhhssgvdlgtenlyfqsMVCEVDP
ELTEKLRKFRFRKETDNAAIIMKVDKDRQ
MVVLEEEFQNISPEELKMELPERQPRFVV
YSYKYVHDDGRVSYPLCFIFSSPVGCKPE
QQMMYAGSKNRLVQTAELTKVFEIRTTDD
LTEAWLQEKLSF
```

Expression: 10 µl of glycerol stock of host strain BL21(DE3)-R3-pRARE2 was used to inoculate 40 ml of TB (terrific Broth) supplemented with 50 µg/ml kanamycin and 34 µg/ml chloramphenicol. This starter culture was grown overnight at 37°C and used next day to inoculate a 4 L TB (5ml starter culture per litre) containing 50 µg/ml kanamycin. The culture was grown at 37°C until the OD_{600} reached ~1.8. After that the temperature was lowered to 18°C and protein production was induced by addition of 0.1 mM IPTG. The expression was continued overnight at that temperature. The next day cells were harvested by centrifugation at 4000 rpm for 20 minutes at 4°C then the supernatant was discarded and pellets re-suspended in binding buffer and stored at -80°C.

Cell Lysis: Binding Buffer: 50mM HEPES pH 7.5, 500mM NaCl, 5% glycerol, 20 mM Imidazole 7.5, 0.5 mM TCEP,1mM PMSF

Procedure: Frozen cells, previously re-suspended, were thawed, and supplemented with benzonase (25U/ml, 2µl of benzonase per 50ml of buffer).Cells were passed 4 times through an Emulsiflex C5 high-pressure homogeniser, collected and centrifuged for 60 min at 15500rpm (Beckman JLA 16.25).

Column 1: Ni- sepharose

Buffers :
Wash Buffer: 50 mM Hepes pH 7.5, 500 mM NaCl, 5% Glycerol, 40 mM Imidazole pH 7.5, 0.5 mM TCEP;
Elution Buffer: 50 mM Hepes pH 7.5, 500 mM NaCl, 5% Glycerol, 250 mM Imidazole pH 7.5, 0.5 mM TCEP

Procedure: The centrifuged supernatant was loaded onto Ni-sepharose column (3 ml resin / litre culture) pre-equilibrated in Binding Buffer. The column was first washed with 20ml of Binding Buffer, followed by 100 ml of Wash buffer and finally eluted with 2.5ml and additional 2ml of elution buffer. All fractions were analyzed by SDS-PAGE.

Column 2: Gel filtration. Hiload S200 16/60

Gel Filtration Buffer: 10 mM Hepes pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP

Procedure: The eluted fractions from Ni-sepharose were clarified by filtration (Acrodisc filters, 0.2 µm) and then loaded on the gel filtration column pre-equilibrated in GF buffer at 1.2 ml/min. Eluted proteins were collected in 1.8ml fractions and analyzed by SDS-PAGE.

Enzymatic treatment: Fractions containing GMFG were pooled and 1mg of TEV protease was added per 45 mg protein. The digestion was performed overnight at 4°C. The following day protein sample was loaded onto Ni-sepharose column (1ml slurry) pre-equilibrated with GF buffer to remove uncleaved protein. The flow-through and wash fractions were pooled and concentrated using Amicon Ultra-15 concentrators with 10 kDa cutoff.

Concentration: Protein was concentrated to 25 mg/ml and frozen at -80°C. Concentrations were determined from the absorbance at 280 nm using NanoDrop.

Mass spectrometry characterisation: Measured: 16 084 Da (ESI-MS); **Expected:** 16 083 Da

Crystallization: Crystals were grown at 4°C by vapour diffusion in sitting drops mixing protein (25 mg/ml) and well solution containing 2.0 M NaCl and 10% PEG 6K at a protein to precipitant ratio of 1:2. Crystals were cryo-protected using 1.7 M Malonate pH 7.0 and flash cooled in liquid nitrogen.

Data Collection: Resolution (scaled): 1.90 Å; **X-ray source:** Diamond I02