Entry Clone Source: cDNA Library

Entry Clone Accession: n/a

SGC Construct ID: AKT3A-c040

GenBank GI number: gi|4885549

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

Coding DNA sequence:

```
ATGCACCATCATCATCATCATTCTTCTGG
TGTAGATCTGGGTACCGAGAACCTGTACT
TCCAATCCATGAGCGATGTTACCATTGTG
AAAGAAGGTTGGGTTCAGAAGAGGGGAGA
ATATATAAAAAACTGGAGGCCAAGATACT
TCCTTTTGAAGACAGATGGCTCATTCATA
GGATATAAAGAGAAACCTCAAGATGTGGA
TTTACCTTATCCCCTCAACAACTTTTCAG
TGGCAAAATGCCAGTTAATGAAAACAGAA
CGACCAAAGCCAAACACATTTATAATCAG
ATGTCTCCAGTGGACTACTGTTATAGAGA
GAACATTTCATGTAGATACTCCAGAGGAA
AGGGAAGAATGGACAGAAGCTATCCAGGC
TGTAGCAGACAGACTGCAGAGGCAAGAAG
AGGAGAGAATGAAT
```

Tags and additions: Cleavable N-terminal His6 tag.

Host: BL21 (DE3)R3-pRARE2

Expressed protein sequence (tag sequence in lowercase):

```
smSDVTIVKEGWVQKRGEYIKNWRPRYFL
LKTDGSFIGYKEKPQDVDLPYPLNNFSVA
KCQLMKTERPKPNTFIIRCLQWTTVIERT
FHVDTPEEREEWTEAIQAVADRLQRQEEE
RMN
```

Expression strain: BL-21(DE3)-R3-Rosetta (A homemade phage resistant version of BL21(DE3) containing the pRARE2 plasmid from Rosetta II (DE3) cells).

Transformation: The construct DNA was transformed into homemade chemically competent cells of the expression strain by a standard heat shock procedure.

Glycerol stock preparation: A number of colonies from the transformation were used to inoculate 1 ml of LB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day glycerol stocks were prepared from this overnight culture.

Expression: A glycerol stock was used to inoculate 50 ml of LB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day this starter culture was used to inoculate 2x 1L of LB media (18 ml starter culture into each) containing 33 µg/ml kanamycin. After 3 hours the temperature was reduced to 18°C. After a further 30 minutes the cells were induced by the addition of 0.5 mM IPTG. The expression was continued overnight.

Cell harvest: Cells were spun at 6238x g for 10 mins at 4°C. The cells were resuspended in 30 ml of Lysis Buffer with the addition of 0.2 mM PMSF. The resuspended cell pellet was placed in a -80°C freezer.

Lysis Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 5 mM Imidazole pH 7.4, 0.5 mM TCEP

Cell Lysis: The resuspended cell pellet was lysed by sonication. PEI (polyethyleneimine) was added to a final concentration of 0.15 % and the cell debris and precipitated DNA were spun down (17000 rpm, JA17 rotor, 45 min).

Column 1: Ni-NTA (2 ml volume in a gravity-flow column).

Buffers:
Binding Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 5 mM Imidazole pH 7.4, 0.5 mM TCEP
Wash Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 25 mM Imidazole pH 7.4, 0.5 mM TCEP
Elution Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 250 mM Imidazole pH 7.4, 0.5 mM TCEP

Procedure: The clarified cell extract was passed through the column. The column was then washed with Binding Buffer (50 ml) and Wash Buffer (25 ml). The protein was eluted with 10 ml of Elution Buffer.

Column 2: S200 16/60 Gel Filtration.

Buffers:
GF Buffer: 25 mM Hepes pH 7.4, 500 mM NaCl, 0.5 mM TCEP

Procedure: The elution buffer fraction from column 1 was concentrated to 4 ml volume in a 10 kDa MW cutoff spin concentrator. The concentrated sample was injected onto an S200 16/60 column (pre-equilibrated in GF Buffer) at 1.0 ml/min. 1.75 ml fractions were collected.

TEV protease digestion: Gel filtration fractions containing AKT3A PH domain were combined. TEV protease was added and the sample left at 4°C over the weekend.

Rebinding of impurities to Ni-NTA: The protein was passed through a gravity-flow column containing Ni-NTA resin (2 ml resin volume, pre-equilibrated into GF Buffer), eluting with GF buffer containing 10 mM imidazole.

Concentration: The TEV protease cleaved protein was concentrated to 12.5 mg/ml (measured by 280 nm absorbance), distributed into aliquots and frozen at -80°C.

Mass spectrometry characterization (ESI-MS): Measured: 14268.8; **Expected:** 14268.3

Crystallisation: Crystals grew from a 1:1 ratio of protein to precipitant solution (0.2 M proline; 0.1M HEPES pH 7.5; 10% PEG 3350), using the vapour diffusion method. Crystals were cryo-protected by equilibration with 50% PEG3350 mixed 1:1 with reservoir solution and 1µl added into precipitant solution, and then flash frozen in liquid nitrogen.

Data Collection: Resolution: 1.54 Å; **X-ray source:** Diamond Synchrotron, Beamline I04.