Entry Clone Source: MGC

Entry Clone Accession: IMAGE:5173213

SGC Construct ID: ABL2A-c055

GenBank GI number: gi|6382060

Vector: pFB-LIC-Bse. Details [PDF]; Sequence [FASTA] or [GenBank]

Amplified construct sequence:

```
TACTTCCAATCCATGGACAAATGGGAAAT
GGAGCGAACAGATATTACCATGAAGCACA
AACTTGGGGGCGGTCAGTATGGAGAGGTT
TACGTTGGCGTCTGGAAGAAATACAGCCT
TACAGTTGCTGTGAAAACATTGAAGGAAG
ATACCATGGAGGTAGAAGAATTCCTGAAA
GAAGCTGCAGTAATGAAGGAAATCAAGCA
TCCTAATCTGGTACAACTTTTAGGTGTGT
GTACTTTGGAGCCACCATTTTACATTGTG
ACTGAATACATGCCATACGGGAATTTGCT
GGATTACCTCCGAGAATGCAACCGAGAAG
AGGTGACTGCAGTTGTGCTGCTCTACATG
GCCACTCAGATTTCTTCTGCAATGGAGTA
CTTAGAGAAGAAGAATTTCATCCATAGAG
ATCTTGCAGCTCGTAACTGCCTAGTGGGA
GAAAACCATGTGGTAAAAGTGGCTGACTT
TGGCTTAAGTAGATTGATGACTGGAGACA
CTTATACTGCTCATGCTGGAGCCAAATTT
CCTATTAAGTGGACAGCACCAGAGAGTCT
TGCCTACAATACCTTCTCAATTAAATCTG
ACGTCTGGGCTTTTGGGGTATTGTTGTGG
GAAATTGCTACCTATGGAATGTCACCATA
TCCAGGTATTGACCTGTCTCAGGTCTATG
ACCTACTAGAAAAAGGATATCGAATGGAA
CAGCCTGAGGGATGCCCCCCTAAGGTTTA
TGAACTTATGAGAGCATGCTGGAAGTGGA
GCCCTGCCGATAGGCCCTCTTTTGCTGAA
ACACACCAAGCTTTTGAAACCATGTTCCA
TGACTCTTGACAGTAAAGGTGGATA
```

Tags and additions: Cleavable N-terminal His6 tag.

Final protein sequence:

```
mghhhhhhssgvdlgtenlyfq^sMDKWE
MERTDITMKHKLGGGQYGEVYVGVWKKYS
LTVAVKTLKEDTMEVEEFLKEAAVMKEIK
HPNLVQLLGVCTLEPPFYIVTEYMPYGNL
LDYLRECNREEVTAVVLLYMATQISSAME
YLEKKNFIHRDLAARNCLVGENHVVKVAD
FGLSRLMTGDTYTAHAGAKFPIKWTAPES
LAYNTFSIKSDVWAFGVLLWEIATYGMSP
YPGIDLSQVYDLLEKGYRMEQPEGCPPKV
YELMRACWKWSPADRPSFAETHQAFETMF
HDS
```

^ TEV cleave site

Host: Baculo Virus infected Insect cells (High5 cells)

Growth medium, induction protocol:
High five cells were grown in Insect Express Medium . Cells were infected at a density of 2x106/ml with recombinant baculovirus (virus stock P2; 1ml of virus stock/100 ml of cell culture). Cells were shaken at 120 rpm at 27°C in the innova shaker. After 48 hours post-infection the cultures were collected and centrifuged for 10min at 2000rpm. The cell pellet was resuspended in cold PBS and centrifugation was repeated. **Bnding buffer**: 50 mM HEPES pH 7.5; 500 mM NaCl; 5 mM imidazole, 5% glycerol; 0.5 mM TCEP.

Extraction buffer, extraction method: Frozen pellets were thawed and cells lysed using a high pressure cell disrupter. PEI (polyethyleneimine) was added to the lysate to a final concentration of 0.15 % and the lysate was centrifuged at 17,000 rpm for 30 minutes and the supernatant collected for purification.

Column 1: Ni-affinity. Ni-NTA (Qiagen), 5 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer.

Buffers: Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 0.5 mM TCEP; **Wash buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 20 mM Imidazole, 5% glycerol, 0.5 mM TCEP; **Elution buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 300 mM Imidazole , 5% Glycerol, 0.5 mM TCEP.

Procedure: The cleared lysate was loaded by gravity flow on the Ni-NTA column. The column was then washed with 100 ml binding buffer and 100 ml wash buffer at gravity flow. The protein was eluted by gravity flow by applying 5-ml of elution buffer.

Column 2: Size Exclusion Chromatography. Superdex S200 16/60 HiLoad.

Buffers: 10 mM HEPES, pH 7.5; 500 mM NaCl, , 5% Glycerol, 0.5 mM TCEP.

Procedure: The protein was concentrated and applied to an S200 16/60 HiLoad gel filtration column equilibrated in 10 mM HEPES, pH 7.5; 500 mM NaCl, , 5% Glycerol, 0.5 mM TCEP using ÄKTA express system.

Mass spectrometry characterization: The mass of the protein was calculated to be 33502 Da and experimentally determined mass was 33414 Da for the His tag containing protein. It is therefore likely that the difference in Mass is due to removal of the initial Met and Acetylation.

Protein concentration: Protein was concentrated to 4.0 mg/ml using an Amicon 10 kDa cut-off concentrator.

Crystallization: Diffraction quality crystals were grown at 4oC in 100 nl drops from a 1:1 ratio of protein and reservoir solution (20% PEG 3350; 0.1M citrate pH 5.5).

Data Collection: Crystals were cryo-protected using 25% EtGly; **Resolution:** 2.05 Å; **X-ray source:** Diffraction data were collected at the SLS-X10SA.