Entry Clone Source: Origene

Entry Clone Accession: XM_039796 variant

SGC Construct ID: TNIKA-c012

GenBank GI number: gi|29728547

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

Amplified construct sequence:

```
CCATGGGCCACCATCATCATCATCATTCTT
CTGGTGTAGATCTGGGTACCGAGAACCTGT
ACTTCCAATCCATGGCGAGCGACTCCCCGG
CTCGAAGCCTGGATGAAATAGATCTCTCGG
CTCTGAGGGACCCTGCAGGGATCTTTGAAT
TGGTGGAACTTGTTGGAAATGGAACATACG
GGCAAGTTTATAAGGGTCGTCATGTCAAAA
CGGGCCAGCTTGCAGCCATCAAGGTTATGG
ATGTCACAGGGGATGAAGAGGAAGAAATCA
AACAAGAAATTAACATGTTGAAGAAATATT
CTCATCACCGGAATATTGCTACATACTATG
GTGCTTTTATCAAAAAGAACCCACCAGGCA
TGGATGACCAACTTTGGTTGGTGATGGAGT
TTTGTGGTGCTGGCTCTGTCACCGACCTGA
TCAAGAACACAAAAGGTAACACGTTGAAAG
AGGAGTGGATTGCATACATCTGCAGGGAAA
TCTTACGGGGGCTGAGTCACCTGCACCAGC
ATAAAGTGATTCATCGAGATATTAAAGGGC
AAAATGTCTTGCTGACTGAAAATGCAGAAG
TTAAACTAGTGGACTTTGGAGTCAGTGCTC
AGCTTGATCGAACAGTGGGCAGGAGGAATA
CTTTCATTGGAACTCCCTACTGGATGGCAC
CAGAAGTTATTGCCTGTGATGAAAACCCAG
ATGCCACATATGATTTCAAGAGTGACTTGT
GGTCTTTGGGTATCACCGCCATTGAAATGG
CAGAAGGTGCTCCCCCTCTCTGTGACATGC
ACCCCATGAGAGCTCTCTTCCTCATCCCCC
GGAATCCAGCGCCTCGGCTGAAGTCTAAGA
AGTGGTCAAAAAAATTCCAGTCATTTATTG
AGAGCTGCTTGGTAAAGAATCACAGCCAGC
GACCAGCAACAGAACAATTGATGAAGCATC
CATTTATACGAGACCAACCTAATGAGCGAC
AGGTCCGCATTCAACTCAAGGACCATATTG
ATAGAACAAAGAAGAAGCGAGGAGAAAAAG
ATGAGACAGAGTATGAGTACAGTGGATGAC
AGTAAAGGTGGATACGGATCCGAATTCGAG
CTCCGTCGACAAGCTT
```

Tags and additions: mghhhhhhssgvdlgtenlyfq. ^: cleavable N-terminal hexahistidine tag.

Final protein sequence (tag sequence in lowercase):

```
mghhhhhhssgvdlgtenlyfq^SMASDSP
ARSLDEIDLSALRDPAGIFELVELVGNGTY
```

GQVYKGRHVKTGQLAAIKVMDVTGDEEEEI
KQEINMLKKYSHHRNIATYYGAFIKKNPPG
MDDQLWLVMEFCGAGSVTDLIKNTKGNTLK
EEWIAYICREILRGLSHLHQHKVIHRDIKG
QNVLLTENAEVKLVDFGVSAQLDRTVGRRN
TFIGTPYWMAPEVIACDENPDATYDFKSDL
WSLGITAIEMAEGAPPLCDMHPMRALFLIP
RNPAPRLKSKKWSKKFQSFIESCLVKNHSQ
RPATEQLMKHPFIRDQPNERQVRIQLKDHI
DRTKKKRGEKDETEYEYSG

Host: SF9 Spodoptera frugiperda Insect cells

Growth medium, induction protocol: Cells at the density of 2millions/ml were infected. Cells were harvested by centrifugation at 4500 rpm at 4°C for 15 min. Cell pellets from each flask 1lt were resuspended in 20 ml binding buffer (50 mM Hepes, pH 7.5; 500 mM NaCl; 5% Glycerol; 5 mM imidazole), transferred to 50 ml tubes, and stored at -20°C.

Extraction buffer, extraction method: The frozen cells were thawed and 1/100 mix protease inhibitor SET V was added to the cell suspension. The cells were lysed by homogenization. The cell lysate was spun down by centrifugation at 21K rpm and 4°C for 1 h. The supernatant was recovered for purification.

Column 1: Anion-exchange for Nucleic acid removal with DEAE cellulose (DE52, Whatmann)10 g of resin was suspended in 50 ml 0.3 M NaCl, and then applied onto a 2.5 x 20 cm column. The resin was then equilibrated with 50 ml binding buffer prior to loading the sample.

Column 1 Buffers:
Binding buffer: 50 mM Hepes, pH 7.5; 500 mM NaCl; 5% Glycerol; 5 mM imidazole 0.1mM TCEP
Wash buffer: 50 mM Hepes, pH 7.5; 500 mM NaCl; 5% Glycerol; 25 mM imidazole 0.1mM TCEP

Column 1 Procedure: The supernatant was first applied onto the column by gravity flow, which was followed by a wash with 100 ml wash buffer. The column flow-through and wash was directly applied onto a Ni-IDA column.

Column 2: Ni-Affinity Chromatography. 5 ml of 50 % Ni-NTA slurry was applied onto a 1.5 x 10 cm column. The column was equilibrated with binding buffer (25ml).

Column 2 Buffer:
Binding buffer: 50 mM Hepes, pH 7.5; 500 mM NaCl; 5% Glycerol; 5 mM imidazole 0.1mM TCEP
Wash buffer: 50 mM Hepes, pH 7.5; 500 mM NaCl; 5% Glycerol; 25 mM imidazole 0.1mM TCEP
Elution buffer: 50 mM HEPES, pH 7.5; 500 mM NaCl; 5% Glycerol; 50 to 250 mM imidazole 0.1mM TCEP

Column 2 Procedure: The flow-through from column 1 (DE52) was applied by gravity flow onto the Ni-NTA column. The bound protein was eluted by applying a step gradient of imidazole - using 12 ml portions of elution buffer with increasing concentration of imidazole (50 mM, 100 mM, 150 mM, 250 mM).

Enzymatic treatment: 0.1mg of Precision TEV protease was added to the elute protein to remove the Tag.

Column 3: Size Exclusion Chromatography - S200 HiLoad 16/60 Superdex run on ÄKTA-Express

Column 3 Buffer: Filtration buffer: 150 mM NaCl,25 mM Hepes pH 7.5, 05mM TCEP

Column 3 Procedure: Prior to applying the protein, the S200 16/60 column was washed and equilibrated with gel filtration buffer. The protein was concentrated to 3 ml using an Amicon Ultra-15 filter with a 30 kDa cut-off. The concentrated protein was directly applied onto the equilibrated S200 16/60 column, and run at a flow-rate of 1 ml/min. The protein was eluted at 80 - 95 ml. Fractions containing the protein were pooled together.

Mass spectrometry characterization: The purified protein was homogeneous and had an experimental mass of 37.184 kDa, as expected from primary sequences. Masses were determined by LC-MS, using an Agilent LC/MSD TOF system with reversed-phase HPLC coupled to electrospray ionisation and an orthogonal time-of-flight mass analyser. Proteins were desalted prior to mass spectrometry by rapid elution off a C3 column with a gradient of 5-95% acetonitrile in water with 0.1% formic acid.

Protein concentration: Protein was concentrated to 10 mg/ml using an Amicon 30 kDa cut-off concentrator.

Crystallization: The protein was crystallised in a buffer containing 150 mM Na-malonate (pH 7.0), 25% (w/v) PEG 3350, 5% (v/v) ethylene glycol, 100 mM Bis-Tris-Propane (pH 9.5) at a temperature of 4°C. Furthermore, a 5 µl 50 mM Wee1/Chk1 inhibitor was present in the crystallisation drop as it was added to the protein solution during concentration.

Data Collection: Data of a single crystal was collected at the Swiss Light Source beamline X10A at a wavelength of 0.999 Å.
Phasing: Phasing was done with molecular replacement based on the PDB ID 3COM.