Entry Clone Source: Five Prime

Entry Clone Accession: n/a

SGC Construct ID: ACVR1A-c076

GenBank GI number: gi|4501895

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

Entry clone accession/ sequence:

```
CCATGGGCCACCATCATCATCATCATTCT
TCTGGTGTAGATCTGGGTACCGAGAACCT
GTACTTCCAATCCATGACCACCAATGTTG
GAGACAGCACTTTAGCAGATTTATTGGAT
CATTCGTGTACATCAGGAAGTGGCTCTGG
TCTTCCTTTTCTGGTACAAAGAACAGTGG
CTCGCCAGATTACACTGTTGGAGTGTGTC
GGGAAAGGCAGGTATGGTGAGGTGTGGAG
GGGCAGCTGGCAAGGGGAAAATGTTGCCG
TGAAGATCTTCTCCTCCCGTGATGAGAAG
TCATGGTTCAGGGAAACGGAATTGTACAA
CACTGTGATGCTGAGGCATGAAAATATCT
TAGGTTTCATTGCTTCAGACATGACATCA
AGACACTCCAGTACCCAGCTGTGGTTAAT
TACACATTATCATGAAATGGGATCGTTGT
ACGACTATCTTCAGCTTACTACTCTGGAT
ACAGTTAGCTGCCTTCGAATAGTGCTGTC
CATAGCTAGTGGTCTTGCACATTTGCACA
TAGAGATATTTGGGACCCAAGGGAAACCA
GCCATTGCCCATCGAGATTTAAAGAGCAA
AAATATTCTGGTTAAGAAGAATGGACAGT
GTTGCATAGCAGATTTGGGCCTGGCAGTC
ATGCATTCCCAGAGCACCAATCAGCTTGA
TGTGGGGAACAATCCCCGTGTGGGCACCA
AGCGCTACATGGCCCCCGAAGTTCTAGAT
GAAACCATCCAGGTGGATTGTTTCGATTC
TTATAAAAGGGTCGATATTTGGGCCTTTG
GACTTGTTTTGTGGGAAGTGGCCAGGCGG
ATGGTGAGCAATGGTATAGTGGAGGATTA
CAAGCCACCGTTCTACGATGTGGTTCCCA
ATGACCCAAGTTTTGAAGATATGAGGAAG
GTAGTCTGTGTGGATCAACAAAGGCCAAA
CATACCCAACAGATGGTTCTCAGACCCGA
CATTAACCTCTCTGGCCAAGCTAATGAAA
GAATGCTGGTATCAAAATCCATCCGCAAG
ACTCACAGCACTGCGTATCAAAAAGACTT
TGACCAAAATTGATTGACAGTAAAGGTGG
ATACGGATCCGAATTCGAGCTCCGTCGAC
AAGCTT
```

Tags and additions: `mghhhhhhssgvdlgtenlyfq*sM` - cleavable N-terminal hexahistidine tag.

Expressed protein sequence (tag sequence in lowercase):

```
mghhhhhhssgvdlgtenlyfqsMTTNVG
DSTLADLLDHSCTSGSGSGLPFLVQRTVA
RQITLLECVGKGRYGEVWRGSWQGENVAV
KIFSSRDEKSWFRETELYNTVMLRHENIL
GFIASDMTSRHSSTQLWLITHYHEMGSLY
```

DYLQLTTLDTVSCLRIVLSIASGLAHLHI
EIFGTQGKPAIAHRDLKSKNILVKKNGQC
CIADLGLAVMHSQSTNQLDVGNNPRVGTK
RYMAPEVLDETIQVDCFDSYKRVDIWAFG
LVLWEVARRMVSNGIVEDYKPPFYDVVPN
DPSFEDMRKVVCVDQQRPNIPNRWFSDPT
LTSLAKLMKECWYQNPSARLTALRIKKTL
TKID

Host: SF9 *Spodoptera frugiperda* Insect cells

Growth medium, induction protocol: Cells at the density of 2 million/ml were infected. Cells were harvested by centrifugation at 4500 rpm at 4°C for 15 min. Cell pellets from each 1lt flask 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 Calbiochem protease inhibitor SET V was added to the cell suspension. The cells were lysed using an Emulsiflex C3 homogeniser. 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.

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.**

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-IDA slurry was applied onto a 1.5 x 10 cm column. The column was equilibrated with binding buffer (25ml).

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; **Elution buffer:** 50 mM HEPES, pH 7.5; 500 mM NaCl; 5% Glycerol; 50 to 250 mM imidazole, 0.1mM TCEP.

Procedure: The flow-through from column 1 (DE52) was applied by gravity flow onto the Ni-IDA 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 TEV protease was added to the Ni-eluted protein to remove the tag.

Complex Assembly: 5mg of ACVR1A and 5mg of FKBP12 (see below for FKBP12 methods) were incubated at 4°C for 30 minutes.

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

Buffer: Gel Filtration buffer: 300 mM NaCl, 50 mM Hepes pH 7.5, 05mM TCEP.

Procedure: Prior to applying the protein, the S200 16/60 column was washed and equilibrated with gel filtration buffer. The two proteins were mixed and concentrated to 3 ml using an Amicon Ultra-15 filter with a 10 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 spec characterization: The purified protein was homogeneous and had an experimental mass of 37.353 and 12.037 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.

MATERIALS & METHODS FOR FKBP12 PRIOR TO COMPLEX FORMATION

Entry Clone Source: MGC

Entry Clone Accession: IMAGE:3504715

SGC Construct ID: FKBP1AA-c001

GenBank GI number: gi|4503725

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

Entry clone accession/ sequence:
ATGGGAGTGCAGGTGGAAACCATCTCCCC
AGGAGACGGGCGCACCTTCCCCAAGCGCG
GCCAGACCTGCGTGGTGCACTACACCGGG
ATGCTTGAAGATGGAAAGAAATTTGATTC
CTCCCGGGACAGAAACAAGCCCTTTAAGT
TTATGCTAGGCAAGCAGGAGGTGATCCGA
GGCTGGGAAGAAGGGGTTGCCCAGATGAG
TGTGGGTCAGAGAGCCAAACTGACTATAT
CTCCAGATTATGCCTATGGTGCCACTGGG
CACCCAGGCATCATCCCACCACATGCCAC
TCTCGTCTTCGATGTGGAGCTTCTAAAAC
TGGAATGA

Tags and additions: mhhhhhhssgvdlgtenlyfq*sM - cleavable N-terminal hexahistidine tag.

Expressed protein sequence:
mhhhhhhssgvdlgtenlyfqsMGVQVET
ISPGDGRTFPKRGQTCVVHYTGMLEDGKK
FDSSRDRNKPFKFMLGKQEVIRGWEEGVA
QMSVGQRAKLTISPDYAYGATGHPGIIPP
HATLVFDVELLKLE

Host: BL21(DE3)-R3-pRARE2

Growth medium, induction protocol: A glycerol stock was used to inoculate a 50 ml starter culture containing LB media and 34 µg/ml chloramphenicol and 50 µg/ml kanamycin. The starter culture was grown overnight at 37°C with shaking at 250 rpm. A flask containing 1L LB media with 34 µg/ml chloramphenicol and 50 µg/ml kanamycin was inoculated with 5 ml of the starter culture. The 1L culture was incubated at 37°C with shaking at 180 rpm until an OD600nm ≥ 0.5 was reached. The flasks were then cooled down to 18°C and 0.5 mM IPTG added to induce protein expression overnight. Cells were harvested by centrifugation at 4500 rpm at 4°C for 15 min. The cell pellet was resuspended in 30 ml binding buffer (50 mM Hepes, pH 7.5; 500 mM NaCl; 5% Glycerol; 5 mM imidazole), transferred to a 50 ml tube, and stored at -20°C.

Extraction buffer, extraction method: The frozen cells were thawed and 0.5 mM TCEP and 1 mM PMSF were added to the cell suspension. The cells were lysed by sonication over 12 min with the sonicator pulsing ON for 5 sec and OFF for 15 sec. The cell lysate was spun down by centrifugation at 16.5k rpm and 4°C for 1 h. The supernatant was recovered for purification.

Columns 1 and 2: FKBP12 was purified from the supernatant using the same column 1/column 2 protocol as shown above for ACVR1. The two proteins were mixed as described above before further purification as described above.

Enzymatic treatment: 0.1mg of TEV protease was added to the Ni-eluted protein to remove the tag.
Crystallisation of the ACVR1-FKBP12 complex: Protein was buffered in 50 mM HEPES, pH 7.5, 150 mM NaCl, 10 mM DTT. To this 1mM dorosomorphin was added and the protein concentrated to 10 mg/ml (calculated using an extinction co-efficient of 46870). Crystals were grown at 4°C in 150 nl sitting drops mixing 100 nl protein solution with 50 nl of a reservoir solution containing 30% PEG3350, 0.25 M (NH4)2SO4, 0.1M BisTris pH 6.0. On mounting crystals were cryoprotected with mother liquor plus 20% PEG400 and flash frozen in liquid nitrogen.
Data Collection: Resolution: 2.35 Å resolution; X-ray source: Diamond Light Source, station I02, using monochromatic radiation at wavelength 0.9050 Å