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:

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TACTTCCAATCCATGGACAAATGGGA
AATGGAGCGAACAGATATTACCATGA
AGCACAAACTTGGGGGCGGTCAGTAT
GGAGAGGTTTACGTTGGCGTCTGGAA
GAAATACAGCCTTACAGTTGCTGTGA
AAACATTGAAGGAAGATACCATGGAG
GTAGAAGAATTCCTGAAAGAAGCTGC
AGTAATGAAGGAAATCAAGCATCCTA
ATCTGGTACAACTTTTAGGTGTGTGT
ACTTTGGAGCCACCATTTTACATTGT
GACTGAATACATGCCATACGGGAATT
TGCTGGATTACCTCCGAGAATGCAAC
CGAGAAGAGGTGACTGCAGTTGTGCT
GCTCTACATGGCCACTCAGATTTCTT
CTGCAATGGAGTACTTAGAGAAGAAG
AATTTCATCCATAGAGATCTTGCAGC
TCGTAACTGCCTAGTGGGAGAAAACC
ATGTGGTAAAAGTGGCTGACTTTGGC
TTAAGTAGATTGATGACTGGAGACAC
TTATACTGCTCATGCTGGAGCCAAAT
TTCCTATTAAGTGGACAGCACCAGAG
AGTCTTGCCTACAATACCTTCTCAAT
TAAATCTGACGTCTGGGCTTTTGGGG
TATTGTTGTGGGAAATTGCTACCTAT
GGAATGTCACCATATCCAGGTATTGA
CCTGTCTCAGGTCTATGACCTACTAG
AAAAAGGATATCGAATGGAACAGCCT
GAGGGATGCCCCCCTAAGGTTTATGA
ACTTATGAGAGCATGCTGGAAGTGGA
GCCCTGCCGATAGGCCCTCTTTTGCT
GAAACACACCAAGCTTTTGAAACCAT
GTTCCATGACTCTTGACAGTAAAGGT
GGATA
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Final protein sequence (Tag sequence in lowercase):

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mghhhhhhssgvdlgtenlyfq^sMD
KWEMERTDITMKHKLGGGQYGEVYVG
VWKKYSLTVAVKTLKEDTMEVEEFLK
EAAVMKEIKHPNLVQLLGVCTLEPPF
YIVTEYMPYGNLLDYLRECNREEVTA
VVLLYMATQISSAMEYLEKKNFIHRD
LAARNCLVGENHVVKVADFGLSRLMT
GDTYTAHAGAKFPIKWTAPESLAYNT
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FSIKSDVWAFGVLLWEIATYGMSPYP
GIDLSQVYDLLEKGYRMEQPEGCPPK
VYELMRACWKWSPADRPSFAETHQAF
ETMFHDS

^ TEV cleavage site

Tags and additions: Cleavable N-terminal His6 tag.

Host: Baculo Virus infected Insect cells (High5 cells).

Growth medium, induction protocol: High five cells grown in Insect Express Medium. Cells were infected at a density of $2x10^6$/ml with recombinant baculovirus (virus stock P2; 1ml of virus stock/100ml of cell culture). Cells were shaken at 120rpm 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.
Binding 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 (polyethleneimine) was added to the lysate to a final concentration of 0.15% and the lysate was centrifuged at 17,000rpm for 30 minutes and the supernatant collected for purification.

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

Column 1 Buffer:
Binding buffer: 50 mM HEPES, pH 7.5; 500 mM NaCl; 5% glycerol; 0.5mM TCEP.
Wash buffer: 50 mM HEPES, pH 7.5; 500 mM NaCl; 5% glycerol; 0.5mM TCEP; 20 mM Imidazole.
Elution buffer: 50 mM HEPES, pH 7.5; 500 mM NaCl; 5% glycerol; 0.5mM TCEP; 300 mM Imidazole.

Column 1 Procedure: The cleared lysate was loaded by gravity flow on the Ni-NTA column. The column was then washed with 100ml binding buffer and 100ml wash buffer at gravity flow. The protein was eluted by gravity flow by applying 5ml of elution buffer.

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

SEC-Buffers:
For the gleevec complex (3GVU): 10 mM HEPES, pH 7.5; 500 mM NaCl; 5% Glycerol; 0.5 mM TCEP.
For the triazole-carbothioamide complex (3HMI): 25 mM HEPES, pH 7.5; 300 mM NaCl; 0.5 mM TCEP.

Column 2 Procedure: The protein was concentrated and applied to an S200 16/60 HiLoad gel fitration column equilibrated in SEC-Buffer using ÄKTA express system.

Protein concentration: Protein was concentrated to 4.0mg/ml (gleevec complex; 3GVU) and 8.0mg/ml (triazole-carbothioamide complex; 3HMI) and 10mg/mL for the VX-680 complex, using an Amicon 10kDa cut-off concentrator.

Mass spectrometry characterization: For the Gleevec complex (3GVU), the mass of the protein was calculated to be 33502Da and experimentally determined mass was 33414Da 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. For the triazole-carbothioamide complex (3HMI),

the mass of the protein was calculated to be 30980 Da after His tag cleavage and experimentally determined mass was 30980 Da.

Crystallisation: The ABL2:imatinib complex (3GVU) was crystallised at 4°C in 200nl drops from a 1:1 ratio of ABL2:imatinib (4mg/ml protein containing 1 mM imatinib) and reservoir solution (20% PEG3350, 0.1 M citrate pH 5.5). The ABL2:JNJ-7706621 complex (3HMI) was crystallised at 4°C in 200nl drops from a 3:1 ratio of ABL2:JNJ-7706621 (8 mg/ml protein containing 1 mM JNJ-7706621 (5-Amino-3-((4-(aminosulfonyl)phenyl)amino)-N-(2,6-difluorophenyl)-1H-1,2,4-triazole-1-carbothioamide, Calbiochem product #217714) and reservoir solution (0.1 M lithium sulfate, 0.05 M di-sodium hydrogen phosphate, 0.05 M citric acid, 19% (v/v) PEG1000). The ABL2:VX-680 complex (2XYN) was crystallised at 4°C in 200nl drops from a 2:1 ratio of ABL2:JNJ-7706621 (10 mg/ml protein containing 1 mM VX-680 (GSK487830B)) and reservoir solution (0.8M Sodium succinate).

Data Collection: Crystals were cryo-protected using 25% ethylene glycol for the gleevec complex and 20% PEG300 for the triazole-carbothioamide complex, respectively..
X-ray source: Diffraction data were collected at the SLS-X10SA (gleevec complex; 3GVU) and at the Diamond beam line I03 (triazole-carbothioamide complex (3HMI) and VX-680 (2XYN).
Phasing: 2.05Å (gleevec complex; 3GVU), 1.65Å (triazole-carbothioamide complex; 3HMI), 2.81Å (VX-680 complex).