Entry Clone Source: MGC
SCG Construct ID: DPYSL3A-c010
Vector: pFB-6HZB

Entry Clone DNA Sequence

ATGTCCTACCAAGGCAAGAAGAACAT
CCCGCGGATCACGAGTGACCGTCTCC
TTATCAAGGGAGGCAGAATCGTCAAT
GATGATCAGTCCTTTTATGCTGATAT
TTACATGGAAGATGGCTTAATAAAAC
AAATTGGAGACAATCTGATTGTTCCT
GGAGGAGTGAAGACCATTGAAGCCAA
TGGGAAGATGGTGATCCCTGGAGGCA
TCGATGTCCATACTCACTACCAGATG
CCATATAAGGGAATGACCACAGTAGA
TGACTTCTTCCAAGGGACAAAGGCGG
CTTTAGCAGGTGGCACCACCATGATC
ATTGACCATGTGGTGCCTGAGCCTGA
GTCCAGCCTGACTGAGGCCTATGAGA
AATGGAGAGAGTGGGCTGATGGGAAG
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TCAAGCAGGAAGTGCAGAACCTCATC
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GTGAAGATCGTCTCTGCCAAGAACCA
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TTGAAGGGATGGAGCTGCGCGGGGCT
CCTCTGGTTGTCATCTGCCAGGGCAA
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ACGTGACCCAGGGGGCTGGCCGCTTC
ATACCCTGCAGCCCGTTCTCCGACTA
TGTCTACAAGCGCATTAAAGCACGGA
GGAAGATGGCAGACCTGCATGCCGTC
CCAAGGGGCATGTACGATGGGCCTGT
GTTTGACCTGACCACCACCCCCAAAG
GTGGCACCCCCGCAGGCTCTGCTCGG
GGCTCTCCTACTCGGCCGAACCCACC
TGTGAGGAATCTTCATCAGTCGGGAT
TTAGCCTGTCAGGCACTCAAGTGGAT
GAGGGGGTTCGCTCAGCCAGCAAGCG
CATCGTGGCGCCCCCAGGCGGCCGTT
CTAATATCACATCTCTGAGTTAA

Tag: *N*-terminal His $_6$-Z-TEV

Construct Protein Sequence

mghhhhhhssgvdnkfnkerrrarre
irhlpnlnreqrrafirslrddpsqs
anllaeakklndaqpkgtenlyfq*s
MSYQGKKNIPRITSDRLLIKGGRIVN
DDQSFYADIYMEDGLIKQIGDNLIVP
GGVKTIEANGKMVIPGGIDVHTHYQM
PYKGMTTVDDFFQGTKAALAGGTTMI
IDHVVPEPESSLTEAYEKWREWADGK
SCCDYALHVDITHWNDSVKQEVQNLI
KDKGVNSFMVYMAYKDLYQVSNTELY
EIFTCLGELGAIAQVHAENGDIIAQE
QTRMLEMGITGPEGHVLSRPEELEAE
AVFRAITIASQTNCPLYVTKVMSKSA
ADLISQARKKGNVVFGEPITASLGID
GTHYWSKNWAKAAAFVTSPPLSPDPT
TPDYINSLLASGDLQLSGSAHCTFST
AQKAIGKDNFTAIPEGTNGVEERMSV
IWDKAVATGKMDENQFVAVTSTNAAK
IFNLYPRKGRISVGSDSDLVIWDPDA
VKIVSAKNHQSAAEYNIFEGMELRGA
PLVVICQGKIMLEDGNLHVTQGAGRF
IPCSPFSDYVYKRIKARRKMADLHAV
PRGMYDGPVFDLTTTPKGGTPAGSAR
GSPTRPNPPVRNLHQSGFSLSGTQVD
EGVRSASKRIVAPPGGRSNITSLS

residues in lower case are cleaved by TEV protease

Host: Insect cells (Sf9)

Expression & Harvest

Exponentially growing Sf9 cells (2xÂ 10 ^{6}Â cells/mL) were infected with high titre baculovirus stock (1:100) and incubated in shaker flasks (50 hours, 92 rpm, 27Â°C). Following this, the cell suspensions were centrifuged (15 minutes, 800x g, 4Â°C), and the cell pellets resuspended in PBS. After another centrifugation (15Â minutes, 800xÂ g, 4Â°C), the cell pellets were stored at -80Â°C.

Purification

A 6Â g cell pellet was thawed, resuspended in 40Â mL lysis buffer (50Â mM HEPES/NaOH pHÂ 7.4, 500Â mM NaCl, 20Â mM imidazole, 0.5Â mM TCEP) and sonicated (2 minutes, amplitude 35%, on ice). The cell lysate was cleared by centrifugation (45Â minutes, 100,000xÂ g, 4Â°C). The supernatant was combined with 5Â mL Ni Sepharose and loaded onto a gravity flow column. The resin was rinsed thrice with lysis buffer. Proteins bound to the resin were eluted with 5x 10Â mL elution buffer (50Â mM HEPES/NaOH pHÂ 7.4, 500Â mM NaCl, 300Â mM imidazole, 0.5Â mM TCEP) and diluted with 50Â mL no salt buffer (20Â mM HEPES/NaOH pHÂ 7.4, 0.5Â mM TCEP).

The next purification step was performed using an AKTAprime system combined with a 5Â ml SP Sepharose column. After equilibrating the column with no salt buffer, the sample was loaded. The column was rinsed with 10% high salt buffer (20Â mM HEPES/NaOH pHÂ 7.4, 2.5Â M NaCl, 0.5Â mM TCEP). The protein was eluted by a linear gradient of 10-100% high salt buffer in 150Â mL.

Fractions containing protein were pooled, combined with recombinant TEV protease (mass ratio 1:25) and incubated with rotation (over night, 4Â°C). Following this, 5Â mL Ni Sepharose was added, and the suspension was loaded onto a gravity flow column. The flowthrough was collected and concentrated to 5.2Â mL.

Finally, the protein was purified by gel filtration using an AKTAxpress system with an S200 16/600 column and GF buffer (20Â mM HEPES/NaOH pHÂ 7.4, 500Â mM NaCl, 0.5Â mM TCEP). Fractions containing protein were pooled, concentrated and stored at -80Â°C.

Crystallisation

Crystals grew from a 1:2 ratio of protein solution (12.6Â mg/mL) and precipitant solution (20% PEG 3.3K, 0.1Â M Bis-Tris-propane pHÂ 8.5, 10% Ethylene Glycol, 0.2Â M KSCN), using the vapour diffusion method.

Data Collection

Crystals were cryo-protected by equilibration into precipitant solution containing 25% ethylene glycol and flash frozen in liquid nitrogen. Data was collected at Diamond, beamline I02.