Entry Clone Source: MGC

Entry Clone Accession: BC030775

SGC Construct ID: ERAP1A-c200

GenBank GI number: gi|94818901

Vector: Vector: pFB-CT10HF-LIC. Details [PDF]; Sequence [FASTA] or [GenBank]

Amplified construct sequence:

CTTAAGAAGGAGATATACTATGGTGT
TTCTGCCCCTCAAATGGTCCCTTGCA
ACCATGTCATTTCTACTTTCCTCACT
GTTGGCTCTCTTAACTGTGTCCACTC
CTTCATGGTGTCAGAGCACTGAAGCA
TCTCCAAAACGTAGTGATGGGACACC
ATTTCCTTGGAATAAAATACGACTTC
CTGAGTACGTCATCCCAGTTCATTAT
GATCTCTTGATCCATGCAAACCTTAC
CACGCTGACCTTCTGGGGAACCACGA
AAGTAGAAATCACAGCCAGTCAGCCC
ACCAGCACCATCATCCTGCATAGTCA
CCACCTGCAGATATCTAGGGCCACCC
TCAGGAAGGGAGCTGGAGAGAGGCTA
TCGGAAGAACCCCTGCAGGTCCTGGA
ACACCCCCGTCAGGAGCAAATTGCAC
TGCTGGCTCCCGAGCCCCTCCTTGTC
GGGCTCCCGTACACAGTTGTCATTCA
CTATGCTGGCAATCTTTCGGAGACTT
TCCACGGATTTTACAAAAGCACCTAC
AGAACCAAGGAAGGGGAACTGAGGAT
ACTAGCATCAACACAATTTGAACCCA
CTGCAGCTAGAATGGCCTTTCCCTGC
TTTGATGAACCTGCCTTCAAAGCAAG
TTTCTCAATCAAAATTAGAAGAGAGC
CAAGGCACCTAGCCATCTCCAATATG
CCATTGGTGAAATCTGTGACTGTTGC
TGAAGGACTCATAGAAGACCATTTTG
ATGTCACTGTGAAGATGAGCACCTAT
CTGGTGGCCTTCATCATTTCAGATTT
TGAGTCTGTCAGCAAGATAACCAAGA
GTGGAGTCAAGGTTTCTGTTTATGCT
GTGCCAGACAAGATAAATCAAGCAGA
TTATGCACTGGATGCTGCGGTGACTC
TTCTAGAATTTTATGAGGATTATTTC
AGCATACCGTATCCCCTACCCAAACA
AGATCTTGCTGCTATTCCCGACTTTC
AGTCTGGTGCTATGGAAAACTGGGGA
CTGACAACATATAGAGAATCTGCTCT
GTTGTTTGATGCAGAAAAGTCTTCTG
CATCAAGTAAGCTTGGCATCACAATG
ACTGTGGCCCATGAACTGGCTCACCA
GTGGTTTGGGAACCTGGTCACTATGG

AATGGTGGAATGATCTTTGGCTAAAT
GAAGGATTTGCCAAATTTATGGAGTT
TGTGTCTGTCAGTGTGACCCATCCTG
AACTGAAAGTTGGAGATTATTTCTTT
GGCAAATGTTTTGACGCAATGGAGGT
AGATGCTTTAAATTCCTCACACCCTG
TGTCTACACCTGTGGAAAATCCTGCT
CAGATCCGGGAGATGTTTGATGATGT
TTCTTATGATAAGGGAGCTTGTATTC
TGAATATGCTAAGGGAGTATCTTAGT
GCTGACGCATTTAAAAGTGGTATTGT
ACAGTATCTCCAGAAGCATAGCTATA
AAAATACAAAAAACGAGGACCTGTGG
GATAGTATGGCAAGTATTTGCCCTAC
AGATGGTGTAAAAGGGATGGATGGCT
TTTGCTCTAGAAGTCAACATTCATCT
TCATCCTCACATTGGCATCAGGAAGG
GGTGGATGTGAAAACCATGATGAACA
CTTGGACACTGCAGAAGGGTTTTCCC
CTAATAACCATCACAGTGAGGGGGAG
GAATGTACACATGAAGCAAGAGCACT
ACATGAAGGGCTCTGACGGCGCCCCG
GACACTGGGTACCTGTGGCATGTTCC
ATTGACATTCATCACCAGCAAATCCG
ACATGGTCCATCGATTTTTGCTAAAA
ACAAAAACAGATGTGCTCATCCTCCC
AGAAGAGGTGGAATGGATCAAATTTA
ATGTGGGCATGAATGGCTATTACATT
GTGCATTACGAGGATGATGGATGGGA
CTCTTTGACTGGCCTTTTAAAAGGAA
CACACACAGCAGTCAGCAGTAATGAT
CGGGCGAGTCTCATTAACAATGCATT
TCAGCTCGTCAGCATTGGGAAGCTGT
CCATTGAAAAGGCCTTGGATTTATCC
CTGTACTTGAAACATGAAACTGAAAT
TATGCCCGTGTTTCAAGGTTTGAATG
AGCTGATTCCTATGTATAAGTTAATG
GAGAAAAGAGATATGAATGAAGTGGA
AACTCAATTCAAGGCCTTCCTCATCA
GGCTGCTAAGGGACCTCATTGATAAG
CAGACATGGACAGACGAGGGCTCAGT
CTCAGAGCGAATGCTGCGGAGTCAAC
TACTACTCCTCGCCTGTGTGCACAAC
TATCAGCCGTGCGTACAGAGGGCAGA
AGGCTATTTCAGAAAGTGGAAGGAAT
CCAATGGAAACTTGAGCCTGCCTGTC
GACGTGACCTTGGCAGTGTTTGCTGT
GGGGGCCCAGAGCACAGAAGGCTGGG
ATTTTCTTTATAGTAAATATCAGTTT
TCTTTGTCCAGTACTGAGAAAAGCCA
AATTGAATTTGCCCTCTGCAGAACCC
AAAATAAGGAAAAGCTTCAATGGCTA
CTAGATGAAAGCTTTAAGGGAGATAA
AATAAAAACTCAGGAGTTTCCACAAA

TTCTTACACTCATTGGCAGGAACCCA
GTAGGATACCCACTGGCCTGGCAATT
TCTGAGGAAAAACTGGAACAAACTTG
TACAAAAGTTTGAACTTGGCTCATCT
TCCATAGCCCACATGGTAATGGGTAC
AACAAATCAATTCTCCACAAGAACAC
GGCTTGAAGAGGTAAAAGGATTCTTC
AGCTCTTTGAAAGAAAATGGTTCTCA
GCTCCGTTGTGTCCAACAGACAATTG
AAACCATTGAAGAAAACATCGGTTGG
ATGGATAAGAATTTTGATAAAATCAG
AGTGTGGCTGCAAAGTGAAAAGCTTG
AACGTATGGCAGAGAACCTCTACTTC
CAATCGCACCATCATCACCATCACCA
TCACCACCATGATTACAAGGATGACG
ACGATAAGTGAGGATCC

Final protein sequence (Tag sequence in lowercase):

MVFLPLKWSLATMSFLLSSLLALLTV
STPSWCQSTEASPKRSDGTPFPWNKI
RLPEYVIPVHYDLLIHANLTTLTFWG
TTKVEITASQPTSTIILHSHHLQISR
ATLRKGAGERLSEEPLQVLEHPRQEQ
IALLAPEPLLVGLPYTVVIHYAGNLS
ETFHGFYKSTYRTKEGELRILASTQF
EPTAARMAFPCFDEPAFKASFSIKIR
REPRHLAISNMPLVKSVTVAEGLIED
HFDVTVKMSTYLVAFIISDFESVSKI
TKSGVKVSVYAVPDKINQADYALDAA
VTLLEFYEDYFSIPYPLPKQDLAAIP
DFQSGAMENWGLTTYRESALLFDAEK
SSASSKLGITMTVAHELAHQWFGNLV
TMEWWNDLWLNEGFAKFMEFVSVSVT
HPELKVGDYFFGKCFDAMEVDALNSS
HPVSTPVENPAQIREMFDDVSYDKGA
CILNMLREYLSADAFKSGIVQYLQKH
SYKNTKNEDLWDSMASICPTDGVKGM
DGFCSRSQHSSSSSHWHQEGVDVKTM
MNTWTLQKGFPLITITVRGRNVHMKQ
EHYMKGSDGAPDTGYLWHVPLTFITS
KSDMVHRFLLKTKTDVLILPEEVEWI
KFNVGMNGYYIVHYEDDGWDSLTGLL
KGTHTAVSSNDRASLINNAFQLVSIG
KLSIEKALDLSLYLKHETEIMPVFQG
LNELIPMYKLMEKRDMNEVETQFKAF
LIRLLRDLIDKQTWTDEGSVSERMLR
SQLLLLACVHNYQPCVQRAEGYFRKW
KESNGNLSLPVDVTLAVFAVGAQSTE
GWDFLYSKYQFSLSSTEKSQIEFALC
RTQNKEKLQWLLDESFKGDKIKTQEF
PQILTLIGRNPVGYPLAWQFLRKNWN
KLVQKFELGSSSIAHMVMGTTNQFST
RTRLEEVKGFFSSLKENGSQLRCVQQ
TIETIEENIGWMDKNFDKIRVWLQSE

KLERMaenlyfq^shhhhhhhhhhdy
kddddk

^ TEV cleavage site

Tags and additions: C-terminal, TEV cleavable decahistidine tag and Flag tag.

Host: *Trichoplusia Ni* (High five)

Growth medium, induction protocol: High five cells were grown in Insect Express medium at 27°C. Cells were infected at a density of 2x106/ml with recombinant baculovirus (virus stock P2; 1ml of virus stock/1l of cell culture) Culture was supplemented with FCS to final concentration 1%. 120 hours post-infection the cultures were collected and centrifuged for 30min at 2000rpm. Cellular pellets were discarded and supernatants were used as a source of a protein.

Column 1: Ni-affinity, Ni-sepharose - (GE Healthcare) purification in batch.

Column 1 Buffers:
Wash buffer: 50 mM HEPES, pH 7.5; 500 mM NaCl; 5% glycerol; 10 mM Imidazole; 1 mM PMSF; 0.5 mM TCEP.
Elution buffer: 50 mM HEPES, pH 7.5; 500 mM NaCl; 5% glycerol; 250 mM Imidazole; 0.5 mM TCEP.

Column 1 Procedure: Supernatant was supplemented with Tris buffer pH 8.0 to final concentration 50 mM, NaCl to final concentration 300 mM, and $NiSO_4$ to final concentration 1 mM. Solution was supplemented with PMSF to final concentration 1 mM and 1 tablet of protease inhibitors per 1l of solution. A resin was added and incubation was performed in room temprature for 4 hours. Suspension was loaded on gravity column and after washing with 20 volumes of washing buffer, protein was eluted in 4 elution fractions (10ml each). Protein fractions were analysed by SDS-PAGE.

Column 2: Superdex S200 column, HiPrep 16/60 (Amersham)

GF Buffer: 10 mM HEPES, pH 7.5; 500 mM NaCl; 5% glycerol; 0.5 mM TCEP.

Column 2 Procedure: Target protein containing fractions were concentrated using Amicon Ultra-15 concentrators with 30kDa cut-off, and purified on a gel filtration column (Superdex S200) on an Åkta Express system. Fractions containing protein were analysed by SDS-PAGE.

Protein concentration: Using Amicon Ultra-15 concentrators with 30kDa cutoff, the sample was concentrated to 17.1mg/ml. Concentrations were determined from the absorbance at 280nm using a NanoDrop spectrophotometer.

Mass spectrometry characterization: The calculated mass of the construct was 110554.5Da, and the observed mass (ESI-MS) was 111563Da, suggesting glycosylation of the protein.

Crystallisation: Crystals were grown by vapor diffusion at 20°C in 150nl sitting drops. The drops were prepared by mixing 100nl of protein solution and 50nl of precipitant consisting of 0.1 M HEPES pH 7.5; 25% PEG 3350. Crystals were flash-cooled in liquid nitrogen with 25% glycerol as cryoprotectant.

Data collection:
Resolution: 3Å.
X-ray source: Diamond Light Source beamline I03.