

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active AMPKA1 [11 – 559] + AMPKB2 [1 – 272] + AMPKG1 [1 - 331]

Enzyme description:-

AMPKA1 [11 – 559] + AMPKB2 [1 – 272] + AMPKG3 [1 - 331]

Clone number:- DU 32489

Source:- Recombinant

Expression system:- *E.coli*,

Tag:-

AMPKA1	N-terminal His(6)
AMPKB2	Untagged
AMPKG1	Untagged

Purification method:- Ni²⁺-NTA agarose

Calculated molecular mass:-

[cysteines reduced, methionines have not been oxidised]

Monoisotopic Mass:

63, 590.24 daltons [AMPKA1], 30, 283.23 daltons [AMPKB2], 37, 555.77 daltons [AMPKG1]

Average Mass:

63, 630.62 daltons [AMPKA1], 30302.22 daltons [AMPKB2], 37, 579.39 daltons [AMPKG1]

Theoretical pI:-

AMPKA1 = 7.39

AMPKB2 = 5.99

AMPKG1 = 6.42

Purity:- >80 %

Activation Protocol:- Activated with GST-CaMKK beta [DU 8964]

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

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Substrate:-

AMARA [AMARAASAAALARRR]

Final concentration: 300 μ M

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Clone Data Sheet

AMPKA1 [11 - 559]

Protein AMPKA1 [11 - 559]

Clone number DU 32489

Species Human

Accession number NM_006251.5

Tags N-terminal GST

Bacterially expressed AMPKA1 protein
MHHHHHHATAEKQKHDGRVKIGHYILGDTLGVGTFGKVKVGKHELTGH
KVAVKILNRQKIRSLDVVGKIRREIQNLKLFRRPHI IKLYQVISTPSD
IFVMMEYVSGGELFDYICKNGRLDEKESRRLFQQILSGVDYCHRHMVV
HRDLKPENVLLDAHNAKIADFGLSNMMSDGEFLRTSCGSPNYAAPEV
ISGRLYAGPEVDIWSSGVILYALLCGTLPFDDDHVPTLFKKICDGIFY
TPQYLNPSVISLLKHMLQVDPMKRATIKDIREHEWFKQDLPKYLFPED
PSYSSTMIDDEALKEVCEKFECSEEEVLSCLYNRNHQDPLAVAYHLII
DNRRIMNEAKDFYLATSPDSDLDDHHLTRPHERVPPFLVAETPRARH
TLDELNPQKSKHQGVRKAKWHLGIRSQSRPNDIMAEVCRAIKQLDYEW
KVVNPYYLRVRRKNPVTSTYSKMSLQLYQVDSRTYLLDFRSIDDEITE
AKSGTATPQRSGSVSNYRSCQRSDSDAEAQGKSSEVSLTSSVTSLDSS
PVDLTPRPGSHTIEFFEMCANLIKILAQ

Native sequence Amino acids A11 – Q559 of human AMPKA1.
Residue A8 of the fusion protein is equivalent to A11 of the native enzyme. The His(6) tag is located at residues 2 – 7.

Protease cleavage None

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**Complete
nucleotide sequence
of AMPKA1**

ATGCATCATCACCATCACCATGCGACAGCCGAGAAGCAGAAACACGAC
GGGCGGGTGAAGATCGGCCACTACATTCTGGGTGACACGCTGGGGGTC
GGCACCTTCGGCAAAGTGAAGGTTGGCAAACATGAATTGACTGGGCAT
AAAGTAGCTGTGAAGATACTCAATCGACAGAAGATTTCGGAGCCTTGAT
GTGGTAGGAAAAATCCGCAGAGAAATTCAGAACCCTCAAGCTTTTCAGG
CATCCTCATATAATTAACCTGTACCAGGTCATCAGTACACCATCTGAT
ATTTTCATGGTGATGGAATATGTCTCAGGAGGAGAGCTATTTGATTAT
ATCTGTAAGAATGGAAGGCTGGATGAAAAAGAAAGTCGGCGTCTGTTC
CAACAGATCCTTTCTGGTGTGGATTATTGTACAGGCATATGGTGGTC
CATAGAGATTTGAAACCTGAAAATGTCCTGCTTGATGCACACATGAAT
GCAAAGATAGCTGATTTTGGTCTTTCAAACATGATGTCAGATGGTGAA
TTTTTAAGAACAAGTTGTGGCTCACCCAACCTATGCTGCACCAGAAGTA
ATTTTCAGGAAGATTGTATGCAGGCCCAGAGGTAGATATATGGAGCAGT
GGGGTTATTCTCTATGCTTTATTATGTGGAACCTTCCATTTGATGAT
GACCATGTGCCAACTCTTTTTAAGAAGATATGTGATGGGATCTTCTAT
ACCCCTCAATATTTAAATCCTTCTGTGATTAGCCTTTTGAAACATATG
CTGCAGGTGGATCCCATGAAGAGGGCCACAATCAAAGATATCAGGGAA
CATGAATGGTTTTAAACAGGACCTTCCAAAATATCTCTTTCCTGAGGAT
CCATCATATAGTTCAACCATGATTGATGATGAAGCCTTAAAAGAAGTA
TGTGAAAAGTTTGAGTGCTCAGAAGAGGAAGTTCTCAGCTGTCTTTAC
AACAGAAATCACCAGGATCCTTTGGCAGTTGCCTACCATCTCATAATA
GATAACAGGAGAATAATGAATGAAGCCAAAGATTTCTATTTGGCGACA
AGCCACCTGATTCTTTTCTTGATGATCATCACCTGACTCGGCCCCAT
CCTGAAAGAGTACCATTCTTGGTTGCTGAAACACCAAGGGCACGCCAT
ACCCTTGATGAATTAATCCACAGAAATCCAAACACCAAGGTGTAAGG
AAAGCAAAATGGCATTTAGGAATTAGAAGTCAAAGTCGACCAAATGAT
ATTATGGCAGAAGTATGTAGAGCAATCAAACAATTGGATTATGAATGG
AAGGTTGTAAACCCATATTTATTTGCGTGTACGAAGGAAGAATCCTGTG
ACAAGCACTTACTCCAAAATGAGTCTACAGTTATACCAAGTGGATAGT
AGAACTTATCTACTGGATTTCCGTAGTATTGATGATGAAATTACAGAA
GCCAAATCAGGGACTGCTACTCCACAGAGATCGGGATCAGTTAGCAAC
TATCGATCTTGCCAAAGGAGTGATTCAGATGCTGAGGCTCAAGGAAAA
TCCTCAGAAGTTTCTCTTACCTCATCTGTGACCTCACTTGACTCTTCT
CCTGTTGACCTAACTCCAAGACCTGGAAGTCACACAATAGAATTTTTT
GAGATGTGTGCAAATCTAATTAATAATCTTGCACAAtaagatct

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Clone Data Sheet

AMPKB2 [1 - 272]

Protein AMPKB2 [1 - 272]

Clone number DU 32489

Species Human

Accession number NM_005399.3

Tags None

**Bacterially
expressed
AMPKB2 protein**

**MGNTTSDRVSGERHGAKAARSEGAGGHAPGKEHKIMVGSTDDPSVFSL
PDSKLPGDKEFVSWQODLEDSVKPTQQARPTVIRWSEGGKEVFI
SGSF
NNWSTKIPLIKSHNDFVAILDLPGEHQYKFFVDGQWVHDPSEPVVTS
QLGTINNLIHVKKSDFEVFDALKLDSMESSETSCRDLSSSPGPYGOE
MYAFRSEERFKSPILPPHLLQVILNKDTNISCDPALLPEPNHVMLNH
LYALSIKDSVMVLSATHRYKKKYVTLLYKPI**

Native sequence Amino acids M1 – I272 of human AMPKB2.

Protease cleavage None

**Complete
nucleotide sequence
of AMPKB2**

**ATGGGAAACACCACCAGCGACCGGGTGTCCGGGGAGCGCCACGGCGCC
AAGGCTGCACGCTCCGAGGGCGCAGGCGGCCATGCCCCGGGGAAGGAG
CACAAGATCATGGTGGGGAGTACGGACGACCCAGCGTGTTTCAGCCTC
CCTGACTCCAAGCTCCCTGGGGACAAAGAGTTTGTATCATGGCAGCAG
GATTTGGAGGACTCCGTAAAGCCCACACAGCAGGCCCCGGCCCACTGTT
ATCCGCTGGTCTGAAGGAGGCAAGGAGGTCTTCATCTCTGGGTCCTTC
AACAAATTGGAGCACCAAGATTCCACTGATTAAGAGCCATAATGACTTT
GTTGCCATCCTGGACCTCCCTGAGGGAGAGCACCATAAAGTTCTTT
GTGGATGGACAGTGGGTTTCATGATCCATCAGAGCCTGTGGTTACCAGT
CAGCTTGGCACAATTAACAATTTGATCCATGTCAAGAAATCTGATTTT
GAGGTGTTTCGATGCTTTAAAGTTAGATTCTATGGAAAGTTCTGAGACA
TCTTGATAGAGACCTTTCCAGCTCACCCCGGGCCTTATGGTCAAGAA
ATGTATGCGTTTCGATCTGAGGAAAGATTCAAATCCCCACCCATCCTT
CCTCCTCATCTACTTCAAGTTATTCTTAACAAAGACACTAATATTTCT
TGTGACCCAGCCTTACTCCCTGAGCCCAACCATGTTATGCTGAACCAT
CTCTATGCATTGTCCATTAAGGACAGTGTGATGGTCCTTAGCGCAACC
CATCGCTACAAGAAGAAGTATGTTACTACTCTGCTATACAAGCCATT
tgaagctt**

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Clone Data Sheet

AMPKG1 [1 - 331]

Protein AMPKG1 [1 - 331]

Clone number DU 32489

Species Human

Accession number BC000358.2

Tags None

Bacterially expressed AMPKG1 protein METVISSDSSPAVENEHPQETPESNNSVYTSFMKSHRCYDLIPTSSKL VVFDTSLQVKKAFFALVTNGVRAAPLWDSKKQSFVGMILTITDFINILH RYYKSALVQIYELEEHKIETWREVYLQDSFKPLVCISPNASLFDVSS LIRNKIHRLPVIDPESGNTLYILTHKRILKFLKLFITEFPKPEFMSKS LEELQIGTYANIAMVRTTTPVYVALGIFVQHRVSALPVVDEKGRVVDI YSKFDVINLAAEKTYNNLDVSVTKALQHRSHYFEGVLKCYLHETLETI INRLVEAEVHRLVVVDENDVVKGI VLSLSDILQALVLTGGEKKP

Native sequence Amino acids M1 – P331 of human AMPKG1.

Protease cleavage None

Complete nucleotide sequence of AMPKG1 ATGGAGACGGTCATTTCTTCAGATAGCTCCCCAGCTGTGGAAAATGAG CATCCTCAAGAGACCCCAGAATCCAACAATAGCGTGTATACTTCCTTC ATGAAGTCTCATCGCTGCTATGACCTGATTCCCACAAGCTCCAAATTG GTTGTATTTGATACGTCCCTGCAGGTGAAGAAAGCTTTTTTTGCTTTG GTGACTAACGGTGTACGAGCTGCCCTTTATGGGATAGTAAGAAGCAA AGTTTTGTGGGCATGCTGACCATCACTGATTTTCATCAATATCCTGCAC CGTACTATAAATCAGCCTTGGTACAGATCTATGAGCTAGAAGAACAC AAGATAGAACTTGGAGAGAGGTGTATCTCCAGGACTCCTTTAAACCG CTTGTCTGCATTTCTCCTAATGCCAGCTTGTTTGATGCTGTCTCTTCA TTAATTCGGAACAAGATCCACAGGCTGCCAGTTATTGACCCAGAATCA GGCAATACTTTGTACATCCTCACCCACAAGCGCATTCTGAAGTTCCTC AAATTGTTTATCACTGAGTTCCCCAAGCCAGAGTTCATGTCCAAGTCT CTGGAAGAGCTACAGATTGGCACCTATGCCAATATTGCTATGGTTTCGC ACTACCACCCCGTCTATGTGGCTCTGGGGATTTTTGTACAGCATCGA GTCTCAGCCCTGCCAGTGGTGGATGAGAAGGGGCGTGTGGTGGACATC TACTCCAAGTTTGATGTTATCAATCTGGCAGCAGAAAAGACCTACAAC AACCTAGATGTATCTGTGACTAAAGCCTTGCAACATCGATCACATTAC TTTGAGGGTGTCTCAAGTGCTACCTGCATGAGACTCTGGAGACCATC ATCAACAGGCTAGTGGAAAGCAGAGGTTACCCGACTTGTAGTGGTGGAT GAAAATGATGTGGTCAAGGGAATTGTATCACTGTCTGACATCCTGCAG GCCCTGGTGCTCACAGGTGGAGAGAAGAAGCCctgaggtacc

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