

## *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:-**

Ni<sup>2+</sup>-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 %

**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

**Substrate:-**

SAMS substrate peptide (HMRSAMSGHLVKRR)

Final concentration:

300  $\mu$ 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**  
MHHHHHHATAEKQKHDGRVKIGHYILGDTLGVGTFGKVKVGKHELTGHKVAVKILNRQKIRSLDVVGKIRREIQNLKLFRRPHI IKLYQVISTPSDIFVMVEYVSGGELFDYICKNGRLDEKESRRLFQQILSGVDYCHRHMVVHRDLKPENVLLDAHNAKIADFGLSNMMSDGEFLRTSCGSPNYAAPEVISGRLYAGPEVDIWSSGVILYALLCGTLPFDDDHVPTLFKKICDGIFYTPQYLNPSVISLLKHMLQVDPMKRATIKDIREHEWFKQDLPKYLPEDPSYSSTMIDDEALKEVCEKFECSEEEVLSCLYNRNHQDPLAVAYHLII DNRRIMNEAKDFYLATSPDSDLDDHHLTRPHPERVPPFLVAETPRARHTLDELNPQKSKHQGVRKAKWHLGIRSQSRPNDIMAEVCRAIKQLDYEWKVVNPYYLRVRRKNPVTSTYSKMSLQLYQVDSRTYLLDFRSIDDEITEAKSGTATPQRSGSVSNYRSCQRSDSDAEAQGKSSEVSLTSSVTSLDSSPVDLTPRPGSHTIEFFEMCANLIKILAQ

**Native sequence** Amino acids A11 – Q559 (end) 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  
CATAGAGATTTGAAACCTGAAAATGTCTTGCTTGATGCACACATGAAT  
GCAAAGATAGCTGATTTTGGTCTTTCAAACATGATGTCAGATGGTGAA  
TTTTTAAGAACAAGTTGTGGCTCACCCAACATGCTGCACCAGAAGTA  
ATTTTCAGGAAGATTGTATGCAGGCCAGAGGTAGATATATGGAGCAGT  
GGGTTATTCTCTATGCTTTATTATGTGGAACCTTCCATTTGATGAT  
GACCATGTGCCAACTCTTTTAAAGAAGATATGTGATGGGATCTTCTAT  
ACCCCTCAATATTTAAATCCTTCTGTGATTAGCCTTTTGAAACATATG  
CTGCAGGTGGATCCCATGAAGAGGGCCACAATCAAAGATATCAGGGAA  
CATGAATGGTTTTAAACAGGACCTTCCAAAATATCTCTTTCTGAGGAT  
CCATCATATAGTTCAACCATGATTGATGATGAAGCCTTAAAAGAAGTA  
TGTGAAAAGTTTGAGTGCTCAGAAGAGGAAGTTCTCAGCTGTCTTTAC  
AACAGAAATCACCAGGATCCTTTGGCAGTTGCCTACCATCTCATAATA  
GATAACAGGAGAATAATGAATGAAGCCAAAGATTTCTATTTGGCGACA  
AGCCACCTGATTCCTTTCTTGATGATCATCACCTGACTCGGCCCCAT  
CCTGAAAGAGTACCATTCTTGGTTGCTGAAACACCAAGGGCACGCCAT  
ACCCCTTGATGAATTAATCCACAGAAATCCAAACACCAAGGTGTAAGG  
AAAGCAAAATGGCATTTAGGAATTAGAAGTCAAAGTCGACCAAATGAT  
ATTATGGCAGAAGTATGTAGAGCAATCAAACAATTGGATTATGAATGG  
AAGGTTGTAAACCCATATTATTTGCGTGTACGAAGGAAGAATCCTGTG  
ACAAGCACTTACTCCAAAATGAGTCTACAGTTATACCAAGTGGATAGT  
AGAACTTATCTACTGGATTTCCGTAGTATTGATGATGAAATTACAGAA  
GCCAAATCAGGGACTGCTACTCCACAGAGATCGGGATCAGTTAGCAAC  
TATCGATCTTGCCAAAGGAGTGATTCAGATGCTGAGGCTCAAGGAAAA  
TCCTCAGAAGTTTCTTTACCTCATCTGTGACCTCACTTGACTCTTCT  
CCTGTTGACCTAACTCCAAGACCTGGAAGTCACACAATAGAATTTTTT  
GAGATGTGTGCAAATCTAATTAATTTCTTGCACAAtaagatct

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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  
PDSKLPGDKEFVSWQODLEDSVKPTQQRPTVIRWSEGGKEVFI  
SGSFNNWSTKIPLIKSHNDFVAILDLPGEHQYKFFVDGQWVHDPSEPVVTS  
QLGTINNLIHVKKSDFEVFDALKLDSMESSETSCRDLSSSPGPYGOE  
MYAFRSEERFKSPILPPHLLQVILNKDTNISCDPALLPEPNHVMLNH  
LYALSIKDSVMVLSATHRYKKKYVTLLYKPI**

**Native sequence** Amino acids M1 – I272 (end) of human AMPKB2.

**Protease cleavage** None

**Complete nucleotide sequence of AMPKB2** **ATGGGAAACACCACCAGCGACCGGGTGTCCGGGGAGCGCCACGGCGCC  
AAGGCTGCACGCTCCGAGGGCGCAGGCGGCCATGCCCCGGGAAGGAG  
CACAAGATCATGGTGGGGAGTACGGACGACCCAGCGTGTTTCAGCCTC  
CCTGACTCCAAGCTCCCTGGGGACAAAGAGTTTGTATCATGGCAGCAG  
GATTTGGAGGACTCCGTAAAGCCCACACAGCAGGCCCGGCCCACTGTT  
ATCCGCTGGTCTGAAGGAGGCAAGGAGGTCTTCATCTCTGGGTCCTTC  
AACAAATTGGAGCACCAAGATTCCACTGATTAAGAGCCATAATGACTTT  
GTTGCCATCCTGGACCTCCCTGAGGGAGAGCACCATAAAGTTCTTT  
GTGGATGGACAGTGGGTTTCATGATCCATCAGAGCCTGTGGTTACCAGT  
CAGCTTGGCACAATTAACAATTTGATCCATGTCAAGAAATCTGATTTT  
GAGGTGTTTCGATGCTTTAAAGTTAGATTCTATGGAAAGTTCTGAGACA  
TCTTGATAGAGACCTTTCCAGCTCACCCCGGGCCTTATGGTCAAGAA  
ATGTATGCGTTTCGATCTGAGGAAAGATTCAAATCCCCACCCATCCTT  
CCTCCTCATCTACTTCAAGTTATTCTTAACAAAGACACTAATATTTCT  
TGTGACCCAGCCTTACTCCCTGAGCCCAACCATGTTATGCTGAACCAT  
CTCTATGCATTGTCCATTAAGGACAGTGTGATGGTCCTTAGCGCAACC  
CATCGCTACAAGAAGAAGTATGTTACTACTCTGCTATAACAAGCCATT  
tgaaagctt**

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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 (end) of human AMPKG1.

**Protease cleavage** None

**Complete nucleotide sequence of AMPKG1** ATGGAGACGGTCATTTCTTCAGATAGCTCCCCAGCTGTGGAAAATGAG CATCCTCAAGAGACCCAGAATCCAACAATAGCGTGTATACTTCCTTC ATGAAGTCTCATCGCTGCTATGACCTGATTCACACAAGCTCCAAATTG GTTGTATTTGATACGTCCCTGCAGGTGAAGAAAGCTTTTTTTGCTTTG GTGACTAACGGTGTACGAGCTGCCCTTTATGGGATAGTAAGAAGCAA AGTTTTGTGGGCATGCTGACCATCACTGATTTTCATCAATATCCTGCAC CGTACTATAAATCAGCCTTGGTACAGATCTATGAGCTAGAAGAACAC AAGATAGAACTTGGAGAGAGGTGTATCTCCAGGACTCCTTTAAACCG CTTGTCTGCATTTCTCCTAATGCCAGCTTGTTTGATGCTGTCTCTTCA TTAATTCGGAACAAGATCCACAGGCTGCCAGTTATTGACCCAGAATCA GGCAATACTTTGTACATCCTCACCCACAAGCGCATTCTGAAGTTCCTC AAATTGTTTATCACTGAGTTCACCAAGCCAGAGTTCATGTCCAAGTCT CTGGAAGAGCTACAGATTGGCACCTATGCCAATATTGCTATGGTTTCGC ACTACCACCCCGTCTATGTGGCTCTGGGGATTTTTGTACAGCATCGA GTCTCAGCCCTGCCAGTGGTGGATGAGAAGGGGCGTGTGGTGGACATC TACTCCAAGTTTGATGTTATCAATCTGGCAGCAGAAAAGACCTACAAC AACCTAGATGTATCTGTGACTAAAGCCTTGCAACATCGATCACATTAC TTTGAGGGTGTCTCAAGTGCTACCTGCATGAGACTCTGGAGACCATC ATCAACAGGCTAGTGGAAAGCAGAGGTTACCCGACTTGTAGTGGTGGAT GAAAATGATGTGGTCAAGGGAATTGTATCACTGTCTGACATCCTGCAG GCCCTGGTGCTCACAGGTGGAGAGAAGAAGCCctgaggtacc

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