

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active AMPKA2 [2 – 552] + AMPKB1 [1 – 270] + AMPKG1 [1 - 331]

Enzyme description:-

AMPKA2 [2 – 552] + AMPKB1 [1 – 270] + AMPKG3 [1 - 331]

Clone number:-

DU 46962

Source:-

Recombinant

Expression system:-

E.coli,

Tag:-

AMPKA2 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, 101.92 daltons [AMPKA2], 30, 363.27 daltons [AMPKB1], 37, 555.77 daltons [AMPKG1]

Average Mass:

63, 142.46 daltons [AMPKA2], 30382.32 daltons [AMPKB1], 37, 579.39 daltons [AMPKG1]

Theoretical pI:-

AMPKA2 = 7.69

AMPKB1 = 5.94

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

AMPKA2 [2 - 552]

Protein AMPKA2 [2 - 552]

Clone number DU 46962

Species Human

Accession number NM_006252.3

Tags N-terminal GST

**Bacterially
expressed**

AMPKA2 protein

MHHHHHHAEKQKHDGRVKIGHYVLGDTLGVGTFGKVKIGEHQLTGHKV
AVKILNRQKIRSLDVVGKIKREIQNLKLFRRPHI IKLYQVISTPTDFF
MVMEYVSGGELFDYICKHGRVEEMEARRLFQQILSAVDYCHRHMVVHR
DLKPENVLLDAHNAKIADFGLSNMMSDGEFLRTSCGSPNYAAPEVIS
GRLYAGPEVDIWSGVIYALLCGTLPFDDEHVPTLFKKIRGGVFYIP
EYLNRSVATLLMHMLQVDPLKRATIKDIREHEWFKQDLPSYLFPEPDS
YDANVIDDEAVKEVCEKFECTESEVMNSLYSGDPQDQLAVAYHLI IDN
RRIMNQASEFYLASSPPSGSFMDDSAMHIPGLKPHPERMPPLIADSP
KARCPLDALNTTKPKSLAVKKAKWHLGIRSQSKPYDIMADEVYRAMKQL
DFEWKVVNAYHLRVRKPNVPTGNYVKMSLQLYLVDNRSYLLDFKSIDD
EVVEQRSGSSTPQRS CSAAGLHRPRSSFDSTTAESHSLSGSLTGSLTG
STLSSVSPRLGSH TMDF FEMCASLITTLAR

Native sequence Amino acids A2 – R552 of human AMPKA2.
Residue A8 of the fusion protein is equivalent to A2 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
AMPKA2**

ATGCATCATCACCATCACCATGCTGAGAAGCAGAAGCACGACGGGCGG
GTGAAGATCGGACACTACGTGCTGGGCGACACGCTGGGCGTTCGGCACC
TTCGGCAAAGTGAAGATTGGAGAACATCAATTAACAGGCCATAAAGTG
GCAGTTAAAATCTTAAATAGACAGAAGATTCGCAGTTTAGATGTTGTT
GGAAAAATAAAACGAGAAATTCAAATCTAAAACCTTTTCGTCATCCT
CATATTATCAAACATAACCAGGTGATCAGCACTCCAACAGATTTTTTT
ATGGTAATGGAATATGTGTCTGGAGGTGAATTAATTTGACTACATCTGT
AAGCATGGACGGGTTGAAGAGATGGAAGCCAGGCGGCTCTTTCAGCAG
ATTCTGTCTGCTGTGGATTACTGTCATAGGCATATGGTTGTTTCATCGA
GACCTGAAACCAGAGAATGTCCTGTTGGATGCACACATGAATGCCAAG
ATAGCCGATTTTCGGATTATCTAATATGATGTCAGATGGTGAATTTCTG
AGAAGTAGTTGCGGATCTCCAAATTATGCAGCACCTGAAGTCATCTCA
GGCAGATTGTATGCAGGTCCTGAAGTTGATATCTGGAGCTGTGGTGT
ATCTTGTATGCTCTTCTTTGTGGCACCCCTCCCATTTGATGATGAGCAT
GTACCTACGTTATTTAAGAAGATCCGAGGGGGTGTCTTTTATATCCCA
GAATATCTCAATCGTTCTGTGCGCCACTCTCCTGATGCATATGCTGCAG
GTTGACCCACTGAAACGAGCAACTATCAAAGACATAAGAGAGCATGAA
TGGTTTAAACAAGATTTGCCAGTTACTTATTTCTGAAGACCCCTTCC
TATGATGCTAACGTCATTGATGATGAGGCTGTGAAAGAAGTGTGTGAA
AAATTTGAATGTACAGAATCAGAAGTAATGAACAGTTTATATAGTGGT
GACCCTCAAGACCAGCTTGCAGTGGCTTATCATCTTATCATTGACAAT
CGGAGAATAATGAACCAAGCCAGTGAGTTCTACCTCGCCTCTAGTCCT
CCATCTGGTTCTTTTATGGATGATAGTGCCATGCATATTCACCCAGGC
CTGAAACCTCATCCAGAAAGGATGCCACCTCTTATAGCAGACAGCCCC
AAAGCAAGATGTCCATTGGATGCACTGAATACGACTAAGCCCAAATCT
TTAGCTGTGAAAAAGCCAAGTGGCATCTTGAATCCGAAGTCAGAGC
AAACCGTATGACATTATGGCTGAAGTTTACCGAGCTATGAAGCAGCTG
GATTTTGAATGGAAGGTAGTGAATGCATAACCATCTTCGTGTAAGAAGA
AAAAATCCAGTGACTGGCAATTACGTGAAAATGAGCTTACAACCTTTAC
CTGGTTGATAACAGGAGCTATCTTTTGGACTTTAAAAGCATTGATGAT
GAAGTAGTGGAGCAGAGATCTGGTTCCTCAACACCTCAGCGTTCCTGT
TCTGCTGCTGGCTTACACAGACCAAGATCAAGTTTTGATTCACAACCT
GCAGAGAGCCATTCACCTTCTGGCTCTCTCACTGGCTCTTTGACCGGA
AGCACATTGTCTTCAGTTTCACCTCGCCTGGGCAGTCACACCATGGAT
TTTTTTGAAATGTGTGCCAGTCTGATTACTACTTTAGCCCGTtgagaa
tcc

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Clone Data Sheet

AMPKB1 [1 - 270]

Protein AMPKB1 [1 - 270]

Clone number DU 46962

Species Human

Accession number NM_006253.4

Tags None

**Bacterially
expressed
AMPKB1 protein**

**MGNTSSERAALERHGGHKTPRRDSSGGTKDGDPRKILMDSPEADLFFH
SEEEKAPEKEEFLLAWQHDLEVNDKAPAQARPTVFRWTGGGKEVYLSGS
FNNWSKLP LTRSHNNFVAI LDLP EGEHQYKFFVDGQWTHDPSEPIVTS
QLGTVNNIIQVKKTD FEVFDALMVDSQKCS DVSELSSSPPGPYHQEPY
VCKPEERFRAPPILP PHL LQVILNKDTGISCDPALLPEPNHVMLNHL
YALS IKDGMVLSATHRYKKKYVTLLYKPI**

Native sequence Amino acids M1 – I270 of human AMPKB1.

Protease cleavage None

**Complete
nucleotide sequence
of AMPKB1**

ATGGGCAATACGAGCAGCGAGCGCGCCGCGCTGGAGCGGCATGGTGGC
CATAAGACGCCCCGAGGGACAGCTCGGGGGGCACCAAGGACGGGGAC
AGGCCAAGATCCTGATGGACAGCCCCGAAGACGCCGACCTCTTCCAC
TCCGAGGAAATCAAGGCACCAGAGAAGGAGGAGTTCTGGCCTGGCAG
CATGATCTGGAAGTGAATGATAAAGCTCCCGCCAGGCTCGGCCAACG
GTGTTTTGATGGACGGGGGGCGGAAAGGAAGTTTACTTATCTGGGTCC
TTCAACAACCTGGAGTAACTTCCCCTCACCAGAAGCCACAATAACTTT
GTAGCCATCCTGGATCTGCCGGAAGGAGAGCATCAGTACAAGTTCTTT
GTGGATGGTCAGTGGACGCACGACCCTCCGAGCCATAGTAACCAGC
CAGCTTGGCACAGTTAACAACATCATTCAAGTGAAGAAAACCTGACTTT
GAAGTATTTGATGCTTTAATGGTGGATTCCCAAAGTGCTCCGATGTG
TCTGAGCTGTCCAGTTCTCCCCAGGACCCTACCATCAGGAGCCCTAC
GTCTGCAAACCCGAAGAGCGCTTTCGGGCACCCCCTATTCTCCCCCA
CATCTCCTCCAGGTCATCCTGAACAAGGACACGGGGATTTCCTGTGAT
CCAGCTTTGCTTCCTGAGCCCAATCACGTCATGCTGAACCACCTATAC
GCGCTGTCTATCAAGGATGGAGTGATGGTGCTCAGCGCAACCCACCG
TACAAGAAGAAGTACGTCACCACCCTCCTCTACAAGCCATAtgaaag
ctt

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Clone Data Sheet

AMPKG1 [1 - 331]

Protein AMPKG1 [1 - 331]

Clone number DU 46962

Species Human

Accession number BC000358.2

Tags None

**Bacterially
expressed
AMPKG1 protein**

**METVISSDSSPAVENEHPQETPESNNSVYTSFMKSHRCYDLIPTSSKL
VVFDTSLQVKKAFFALVTNGVRAAPLWDSKKQSFVGLTITDFINILH
RYYKSALVQIYELEEHKIETWREVYLQDSFKPLVCISPNASLFDVSS
LIRNKIHRPLVIDPESGNTLYILTHKRILKFLKLFITEFPKPEFMSKS
LEELQIGTYANIAMVRTTTPVYVALGIFVQHRVSALPVVDEKGRVVDI
YSKFDVINLAAEKTYNNLDVSVTKALQHRSHYFEGVLKCYLHETLETI
INRLVEAEVHRLVVVDENDVVKGI VLSLSDILQALVLTGGEKKP**

Native sequence Amino acids M1 – P331 of human AMPKG1.

Protease cleavage None

**Complete
nucleotide sequence
of AMPKG1**

**ATGGAGACGGTCATTTCTTCAGATAGCTCCCCAGCTGTGGAAAATGAG
CATCCTCAAGAGACCCAGAATCCAACAATAGCGTGTATACTTCCTTC
ATGAAGTCTCATCGCTGCTATGACCTGATTCACACAAGCTCCAAATTG
GTTGTATTTGATACGTCCCTGCAGGTGAAGAAAGCTTTTTTTGCTTTG
GTGACTAACGGTGTACGAGCTGCCCTTTATGGGATAGTAAGAAGCAA
AGTTTTGTGGGCATGCTGACCATCACTGATTTTCATCAATATCCTGCAC
CGCTACTATAAATCAGCCTTGGTACAGATCTATGAGCTAGAAGAACAC
AAGATAGAAACTTGGAGAGAGGTGTATCTCCAGGACTCCTTTAAACCG
CTTGTCTGCATTTCTCCTAATGCCAGCTTGTGGTATGCTGTCTCTTCA
TTAATTCGGAACAAGATCCACAGGCTGCCAGTTATTGACCCAGAATCA
GGCAATACTTTGTACATCCTCACCCACAAGCGCATTCTGAAGTTCCTC
AAATTGTTTATCACTGAGTTCACCAAGCCAGAGTTCATGTCCAAGTCT
CTGGAAGAGCTACAGATTGGCACCTATGCCAATATTGCTATGGTTCCG
ACTACCACCCCGTCTATGTGGCTCTGGGGATTTTTGTACAGCATCGA
GTCTCAGCCCTGCCAGTGGTGGATGAGAAGGGCGTGTGGTGGACATC
TACTCCAAGTTTGATGTTATCAATCTGGCAGCAGAAAAGACCTACAAC
AACCTAGATGTATCTGTGACTAAAGCCTTGCAACATCGATCACATTAC
TTTGAGGGTGTCTCAAGTGCTACCTGCATGAGACTCTGGAGACCATC**

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ATCAACAGGCTAGTGGAAGCAGAGGTTACCGACTTGTAGTGGTGGAT
GAAAATGATGTGGTCAAGGGAATTGTATCACTGTCTGACATCCTGCAG
GCCCTGGTGCTCACAGGTGGAGAGAAGAAGCCctgaggtacc