AMPKA2 [6His-tagged]/AMPKB2/AMPKG1

Kinase

Alternate Names: AMPKA2 = 5'-AMP-activated protein kinase catalytic subunit alpha-2, AMPK subunit alpha-2; AMPKB2 = 5'-AMP-activated protein kinase subunit beta-2, AMPK subunit beta-2; AMPKG1 = 5'-AMP-activated protein kinase subunit gamma-1, Short name=AMPK subunit gamma-1

Cat. No. **66-0042-050**
Lot. No. **30321**

Quantity: 50 µg
Storage: -70˚C

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NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

Background

Protein ubiquitylation and protein phosphorylation are the two major mechanisms that regulate the functions of proteins in eukaryotic cells. However, these different posttranslational modifications do not operate independently of one another, but are frequently interlinked to enable biological processes to be controlled in a more complex and sophisticated manner. Studying how protein phosphorylation events control the ubiquitin system and how ubiquitylation regulates protein phosphorylation has become a focal point of the study of cell regulation and human disease. Cloning of human 5'-AMP-activated protein kinase subunits alpha, beta and gamma (AMPKαβγ) was first described by Stapleton *et al.* (1996; 1997). AMPK is a highly conserved heterotrimeric enzyme consisting of three subunits, α, β, and γ, with multiple genes encoding distinct subunit isoforms (ie, α1, α2, β1, β2, γ1, γ2, and γ3) (Zungu *et al.*, 2011). AMPK consists of an α, catalytic subunit (63 kDa) and non-catalytic, ß (40 kDa) and γ (38 kDa) subunits. Co-expression of the non-catalytic ß and γ subunits is required for optimal activity of the α catalytic subunit (Stapleton *et al.*, 1997). An example of such interplay between phosphorylation and ubiquitylation has been highlighted in recent studies indicating that AMPKα, along with AMPK kinases NUAK1 and MARK4, can be ubiquitylated with atypical ubiquitin chains. The deubiquitylating enzyme (DUB) found to remove these ubiquitin chains from

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Physical Characteristics

Species: human

Source: *E. coli*

Quantity: 50 µg

Concentration: 0.27 mg/ml

Formulation: 50 mM Tris/HCl pH7.5, 0.1 mM EGTA, 150 mM NaCl, 0.1% ß-Mercaptoethanol, 270 mM sucrose, 0.03% Brij-35, 1 mM Benzamidine, 0.2 mM PMSF

Molecular Weight: AMPKA2 ~63.2 kDa; AMPKB2 ~30.3 kDa; AMPKG1 ~37.6 kDa

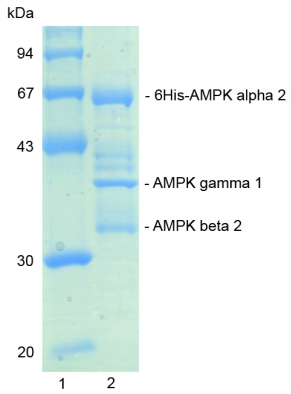
Purity: n/a

Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequences: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 2.5 µg 6His-AMPKA2 /AMPKB2/AMPKG1



Protein Identification:
Confirmed by mass spectrometry.

Activity Assay:
The specific activity of AMPKA2 [6His-tagged]/AMPKB2/AMPKG1 was determined using the method described by Hastie *et al.* (2006) with the enzyme being assayed at several concentrations. AMPKA2 [6His-tagged]/AMPKB2/AMPKG1 was incubated for 10 minutes at 30˚C in kinase reaction buffer in the presence of AMARA peptide substrate (300 µM) and [γ-32P]ATP (100 µM). Duplicate reactions were stopped by spotting the assay mixture onto Whatman P81 paper – capturing the phosphorylated substrate. The radioactivity incorporated was measured on a scintillation counter and the enzyme's mean specific activity was calculated.

AMPKA2 [6His-tagged]/AMPKB2/AMPKG1 specific activity: 4435 Units/mg (1198 Units/ml)

1 Unit = 1 nmole of phosphate incorporated into the substrate in 1 minute

Substrate: AMARA peptide (AMARAASAAALARRR)

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Lot-specific COA version tracker: v1.0.0

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Cat. No.	**66-0042-050**	Quantity:	50 µg
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Background

Continued from page 1

both NUAK1 and MARK4 has been identified as USP9X. AMPK activation has also been shown to increase the expression of the E3 ubiquitin ligases MAFBx/Atrogin-1 and MuRF1. These ubiquitin ligases regulate key cardiac transcription factors to control cardiomyocyte mass and remodeling, thus suggesting another mechanism by which AMPK may function in the heart. The relevance of AMPK ubiquitylation in cardiac disease has yet to be tested directly, but it likely represents an important mechanism that occurs in common cardiac diseases that may be targeted for therapy (Zungu *et al.*, 2011).

References:

Hastie CJ, McLauchlan HJ, Cohen P (2006) Assay of protein kinases using radiolabeled ATP: a protocol. *Nat Protoc* **1**, 968-71.

Stapleton D, Mitchelhill KI, Gao G, Widmer J, Michell BJ, Teh T, *et al.* (1996) Mammalian AMP-activated protein kinase subfamily. *J Biol Chem* **271**, 611-614.

Stapleton D, Woollatt E, Mitchelhill KI, Nicholl JK, Fernandez CS, Michell BJ *et al.* (1997) AMP-activated protein kinase isoenzyme family: subunit structure and chromosomal location. *FEBS Lett* **409**, 452-456.

Zungu M, Schisler JC, Essop MF, McCudden C, Patterson C and Willis MS (2011) Regulation of AMPK by the ubiquitin proteasome system. *Am J Pathol* **178**, 4-11.

Physical Characteristics

Continued from page 1

AMPK alpha 2 Protein Sequence:

MHHHHHHAEKQKHDGRVKIGHYV
LGDTLGVGTFGKVKIGEHQLTGHKVAVKILN
RQKIRSLDVVGKIKREIQNLKLFRHPHIIK
LYQVISTPTDFFMVMEYVSGGELFDYICK
HGRVEEMEARRLFQQILSAVDYCHRHMV
VHRDLKPENVLLDAHMNAKIADFGLSNMMS
DGEFLRTSCGSPNYAAPEVISGRLYAGPE
VDIWSCGVILYALLCGTLPFDDEHVPTLFK
KIRGGVFYIPEYLNRSVATLLMHMLQVD
PLKRATIKDIREHEWFKQDLPSYLFPEDPSY
DANVIDDEAVKEVCEKFECTESEVMNSLYS
GDPQDQLAVAYHLIIDNRRIMNQASEFY
LASSPPSGSFMDDSAMHIPPGLKPHPERMP
PLIADSPKARCPLDALNTTKPKSLAVKKAK
WHLGIRSQSKPYDIMAEVYRAMKQLDFEWKV
VNAYHLRVRRKNPVTGNYVKMSLQLYLVD
NRSYLLDFKSIDDEVVEQRSGSSTPQRSC
SAAGLHRPRSSFDSTTAESHSLSGSLTG
SLTGSTLSSVSPRLGSHTMDFFEMCASLIT
TLAR

Tag (**bold text**): N-terminal 6His
Protease cleavage site: none
AMPK alpha 2 (regular text): Start ***bold italics*** (amino acid residues 2-552).
Accession number: NP_006243

AMPK beta 2 Protein Sequence:

MGNTTSDRVSGERHGAKAARSEGAGGHAPG
KEHKIMVGSTDDPSVFSLPDSKLPGD
KEFVSWQQDLEDSVKPTQQARPTVIRWSEG
GKEVFISGSFNNWSTKIPLIKSHNDFVAILD
LPEGEHQYKFFVDGQWVHDPSEPVVTSQL
GTINNLIHVKKSDFEVFDALKLDSMES
SETSCRDLSSSPPGPYGQEMYAFRSEERFK
SPPILPPHLLQVILNKDTNISCDPALLPEPN
HVMLNHLYALSIKDSVMVLSATHRYKKKYVT
TLLYKPI

Tag: None
Protease cleavage site: none
AMPK beta 2 (regular text): Start ***bold italics*** (amino acid residues 1-272).
Accession number: NP_005390

AMPK gamma 1 Protein Sequence:

METVISSDSSPAVENEHPQETPESNNSVYTS
FMKSHRCYDLIPTSSKLVVFDTSLQVK
KAFFALVTNGVRAAPLWDSKKQSFVGM
LTITDFINILHRYYKSALVQIYELEEHKI
ETWREVYLQDSFKPLVCISPNASLFDAVSS
LIRNKIHRLPVIDPESGNTLYILTHKRILK
FLKLFITEFPKPEFMSKSLEELQIGTYANIA
MVRTTTPVYVALGIFVQHRVSALPVVDEK
GRVVDIYSKFDVINLAAEKTYNNLDVSVTKA
LQHRSHYFEGVLKCYLHETLETIINRLVE
AEVHRLVVVDENDVVKGIVSLSDILQALVLT
GGEKKP

Tag: None
Protease cleavage site: none
AMPK gamma 1 (regular text): Start ***bold italics*** (amino acid residues 1-331).
Accession number: AAH00358

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