AMPK alpha 1 (T172D) [GST-tagged]

Kinase

Alternate Names: 5'-AMP-activated protein kinase catalytic subunit alpha-1, Acetyl-CoA carboxylase kinase, Hydroxymethylglutaryl-CoA reductase kinase, HMGCR kinase, Tau-protein kinase PRKAA1

Cat. No. **66-0040-050**
Lot. No. **30319**

Quantity: 50 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

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 catalytic subunit alpha-1 (AMPK alpha 1) was first described by Stapleton *et al.* (1996). An example of such interplay between phosphorylation and ubiquitylation has been highlighted in recent studies indicating that AMPK alpha, 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 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

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Physical Characteristics

Species: rat

Source: *E. coli*

Quantity: 50 µg

Concentration: 2.0 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: ~64.6 kDa

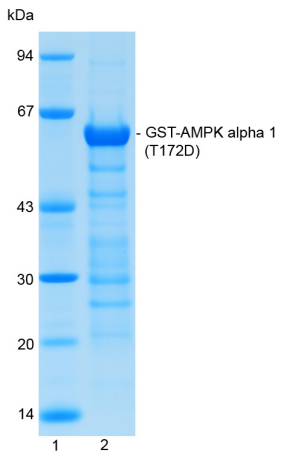
Purity: >95% by InstantBlue™ SDS-PAGE

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

Protein Sequence: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 2.5 µg GST-AMPK alpha 1 (T172D)



Protein Identification:
Confirmed by mass spectrometry.

Activity Assay:
The specific activity of GST-AMPK alpha 1 (T172D) was determined using the method described by Hastie *et al.* (2006) with the enzyme being assayed at several concentrations. GST-AMPK alpha 1 (T172D) 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.

GST-AMPK alpha 1 (T172D) specific activity:
41.73 Units/mg (64.27 Units/ml)

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

Substrate: AMARA peptide (AMARAASAAALARRR)

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Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

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Background

Continued from page 1

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.

Stein SC, Woods A, Jones NA, Davison MD, Carling D (2000) The regulation of AMP-activated protein kinase by phosphorylation. *Biochem J* **345**, 437-443.

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

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKERAEISM
LEGAVLDIRYGVSRIAYSKDFETLKVDFL
SKLPEMLKMFEDRLCHKTYLNGDHVTHPD
FMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQATF
GGGDHPPKSDLVPRGSEFAM**EQKLISEEDL**
GGG***E***KQKHDGRVKIGHYILGDTLGVGTFG
KVKVGKHELTGHKVAVKILNRQKIRSLDV
VGKIRREIQNLKLFRHPHIIKLYQVISTPS
DIFMVMEYVSGGELFDYICKNGRLDEKESR
RLFQQILSGVDYCHRHMVVHRDLKPENVLL
DAHMNAKIADFGLSNMMSDGEFLRDSCGSP
NYAAPEVISGRLYAGPEVDIWSSGVILYALL
CGTLPFDDDHVPTLFKKICDGIFYTPQYL
NPSVISLLKHMLQVDPMKRATIKDIREHEW
FKQDLPKYLFPEDPSYSSTMIDDEALKEVCEK
FECSEEEVL**RSITLAAARDRLD**

Tag (**bold text**): N-terminal GST and N-terminal MYC
Protease cleavage site: Thrombin (LVPR▼GS)
AMPK alpha 1 (regular text): Start ***bold italics*** (amino acid residues 3-308).
Non-AMPK sequence derived from the cloning vector (**bold underlined**).
AMPK alpha 1 has a T172D mutation to mimic the activation of the enzyme through phosphorylation of Thr172 by PDK1 (Stein *et al.*, 2000).
Accession number: AAC52355

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Lot-specific COA version tracker: v1.0.0