AMPK (A1/B1/G1), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SEA11

Lot No.
5 µg 0.1 µg/µl

Background:

AMPK is a heterotrimer protein kinase consisting of α catalytic subunit, and non-catalytic β and γ subunits. AMPK is an important energy-sensing enzyme that monitors cellular energy status (1). In response to cellular metabolic stresses, AMPK is activated and phosphorylates and inactivates acetyl-CoA carboxylase (ACC) and beta-hydroxy beta-methylglutaryl-CoA reductase (HMGCR), key enzymes involved in regulating biosynthesis of fatty acid and cholesterol (2).

Product Description:

Recombinant full-length human AMPK (combination of A1/B1/G1 subunits) was expressed by baculovirus in Sf9 insect cells using a C-terminal His tag. The gene accession numbers for the three subunits (A1/B1/G1) are NM_006251, NM_006253, and NM_002733.

Gene Aliases:

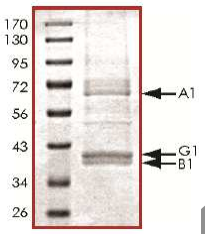
Subunit A1: PRKAA1, MGC33776, MGC57364
Subunit B1: PRKAB1, AMPK, HAMPKb, MGC17785
Subunit G1: PRKAG1, AMPKG, MGC8666

Formulation:

Recombinant protein stored in 50mM sodium phosphate, pH 7.0, 300mM NaCl, 150mM imidazole, 0.1mM PMSF, 0.25mM DTT, 25% glycerol

Purity & Molecular Weight:

The purity of AMPK was determined to be >85% by densitometry.
Approx. MW 68kDa (A1), 38kDa (B1), and 40kDa (G1).



Storage:

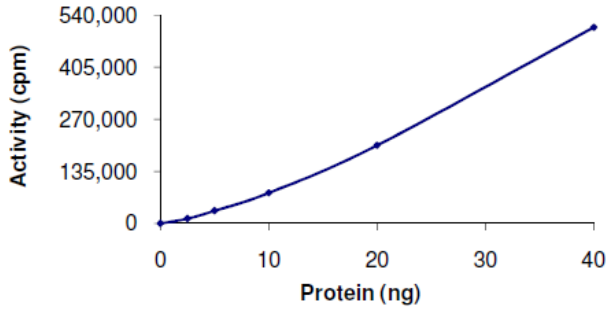
Store product at –70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

Unopened vial at -70 °C, for 1 year after delivery.

Specific Activity:

The specific activity was determined to be 675 nmol /min/mg as per Activity Assay Protocol.



Activity Assay Protocol:

Assay activity of the kinase in a 25 µL reaction consisting of 5 µL of 5 X Kinase Assay Buffer, 5 µL of 1 mg/ml the Substrate Solution, 5 µl of 0.5mM AMP solution, 5 µL of diluted kinase and 5 µL of 250 µM ATP solution containing [gamma ^{32}P] ATP (0.167 µCi/µL). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20 µL of the reaction mixture onto phosphocellulose P81 paper. Air-dry the P81 paper and sequentially wash 4 times for approximately 10 minutes each in 1% phosphoric acid with constant gentle stirring. Count the P81 paper in a liquid scintillation counter.

Substrate Solution:

SAMStide synthetic peptide substrate (HMRSAMSGLHLVKRR) diluted in distilled H_2O to a final concentration of 1mg/ml.

5 X Kinase Assay Buffer:

25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

References:

1. Minokoshi, Y. et al:AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. Nature 428: 569-574, 2004.
2. Hardie, D G. et al: The AMP-activated protein kinase—fuel gauge of the mammalian cell? Eur J Biochem. 1997 Jun 1;246(2):259-73.

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