AMPK (A1/B1/G3), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SEA13

Lot No.
5 µg 0.1 µg/µl

Background:

AMPK (A1/B1/G3) is a member of the AMPK family which are heterotrimeric proteins consisting of α catalytic subunit, and non-catalytic β and γ subunits. AMPKs are an important energy-sensing enzyme group in the cells that monitor energy status particularly in response to stress (1). AMPKs regulate fatty acid and cholesterol synthesis by regulating the key rate-limiting enzymes acetyl-CoA carboxylase and hydroxy betamethylglutaryl-CoA reductase. The γ subunit is dominantly expressed in skeletal muscle where it may play a key role in the regulation of energy metabolism (2).

Product Description:

Recombinant full-length human AMPK (combination of A1/B1/G3 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/G3) are NM_006251, NM_006253, and NM_017431.

Gene Aliases:

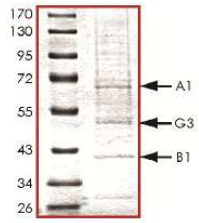
Subunit A1: PRKAA1, MGC33776, MGC57364
Subunit B1: PRKAB1, AMPK, HAMPKb, MGC17785
Subunit G3: PRKAG3

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 >70% by densitometry.
Approx. MW 68kDa (A1), 38kDa (B1), and 51kDa (G3).



Storage:

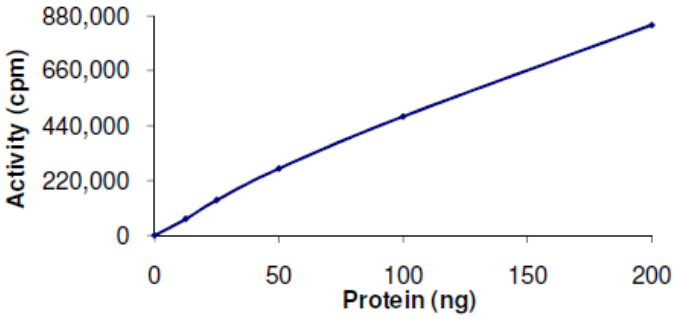
Store product at –70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

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

Specific Activity:

The specific activity was determined to be 212 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. Viollet, B. et al: Physiological role of AMP-activated protein kinase (AMPK): insights from knockout mouse models. Biochem. Soc. Trans. 2003; 31; 216–219.

2. Cheung, P. C. F. et al: Characterization of AMP-activated protein kinase gamma-subunit isoforms and their role in AMP binding. Biochem. J. 346: 659-669, 2000.

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