AMPK (A2/B2/G1), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SEA24

Lot No.
5 µg 0.1 µg/µl

Background:

AMP-activated protein kinase (AMPK) exhibits a key role as a master regulator of cellular energy homeostasis (1). AMPK exists as a heterotrimeric complex composed of a catalytic α subunit and regulatory β and γ subunits. Binding of AMP to the γ subunit allosterically activates the complex. AMPK is activated in response to stresses that deplete cellular ATP (low glucose, hypoxia and ischemia) (2) and via signaling pathways in response to adiponectin, leptin and CAMKKβ.

Product Description:

Recombinant full-length human AMPK (combination of A2/B2/G1 subunits) was expressed by baculovirus in Sf9 insect cells using a C-terminal His tag. The gene accession numbers for the three subunits (A2/B2/G1) are NM_006252, NM_005399, and NM_002733.

Gene Aliases:

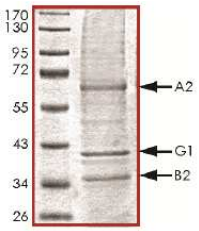
Subunit A2: PRKAA2, AMPK, AMPK2, PRKAA
Subunit B2: PRKAB2, MGC61468
Subunit G1: PRKAG1, AMPKG, MGC8666

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 >75% by densitometry.
Approx. MW 69kDa (A2), 36kDa (B2), and 41kDa (G1).



Storage:

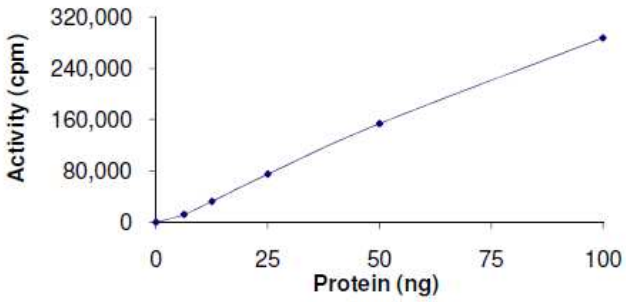
Store product at –70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

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

Specific Activity:

The specific activity was determined to be 240 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. Hardie, G.D. The AMP-activated protein kinase pathway –new players upstream and downstream. J. Cell Sci. 2004;117: 5479–5487.
2. Kahn, B.B. et al. AMP-activated protein kinase: Ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab; 2005: 1, 15–25.

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