



AMPK (A1/B2/G1), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SEA14

Lot No.
5 µg 0.1 µg/µl

Background:

AMPK (A1/B2/G1) 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 β subunit may be a positive regulator of AMPK activity and is highly expressed in skeletal muscle (2).

Product Description:

Recombinant full-length human AMPK (combination of A1/B2/G1 subunits) was expressed by baculovirus in Sf9 insect cells using C-terminal His tags. The gene accession numbers for the three subunits (A1/B2/G1) are NM_006251, NM_005399, and NM_002733.

Gene Aliases:

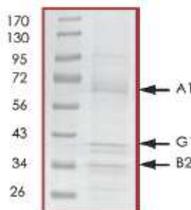
Subunit A1: PRKAA1, MGC33776, MGC57364
Subunit B2: PRKAB2, MGC61468
Subunit G1: PRKAG1, AMPKG, MGC8666

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 >70% by densitometry. Approx. MW 68kDa (A1), 36kDa (B2), and 40kDa (G1).



Storage:

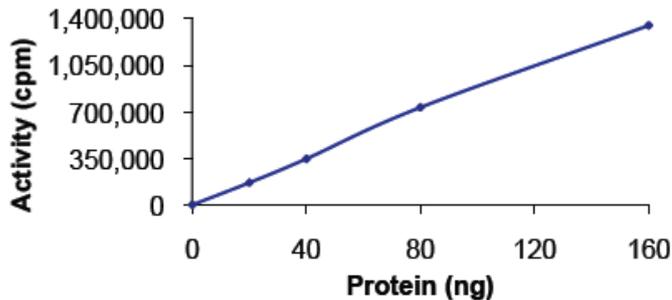
Store product at -70°C . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

**Stability:**

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

Specific Activity:

The specific activity was determined to 390 nmol /min/mg as per Activity Assay Protocol.

**Activity Assay Protocol:**

Assay activity of the kinase in a 25 μ L reaction consisting of 5 μ L of 5 X Kinase Assay Buffer, 5 μ L of 1 mg/ml the Substrate Solution, 5 μ L of 0.5mM AMP solution, 5 μ L of diluted kinase and 5 μ L of 250 μ M ATP solution containing [γ 32 P] ATP (0.167 μ Ci/ μ L). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20 μ L 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 (HMRSAMSGHLVKRR) diluted in distilled H₂O to a final concentration of 1mg/ml.

5 X Kinase Assay Buffer:

25mM MOPS, pH 7.2, 12.5mM β -glycerol-phosphate, 25mM MgCl₂, 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. Thornton, C. et al: Identification of a novel AMP-activated protein kinase beta subunit isoform that is highly expressed in skeletal muscle. *J. Biol. Chem.* 273: 12443-12450, 1998.

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