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.

This product is for research use only and is not intended for use in humans.
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 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 ^32P] 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 (HMRSAMGLHLVKRR) 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:

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