



## AMPK (A2/B1/G1), Active

### Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SEA21

Lot No. Q260-1  
5 µg 0.1 µg/µl

#### Background:

AMPK (A2/B1/G1) plays a key role in insulin signaling pathway and is a major therapeutic target for the treatment of diabetes (1). AMPK is viewed as a fuel sensor for glucose and lipid metabolism by modulating the activity of the autonomous nervous system in vivo. Short-term overexpression of a constitutively active form of AMPK in the liver leads to mild hypoglycemia and fatty liver due to increased fatty acid utilization (2).

#### Product Description:

Recombinant full-length human AMPK (combination of A2/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 (A2/B1/G1) are NM\_006252, NM\_006253, and NM\_002733

#### Gene Aliases:

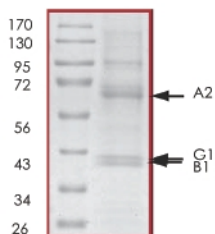
Subunits A2: PRKAA2, AMPK, AMPK2, PRKAA  
Subunit B1: PRKAB1, AMPK, HAMPKb, MGC17785  
Subunit G1: PRKAG1, AMPKG, MGC8666

#### Formulation:

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

#### Purity & Molecular Weight:

The purity of AMPK was determined to be >75% by densitometry.  
Approx. MW 69kDa (A2), 38kDa (B1), and 40kDa (G1).



#### Storage:

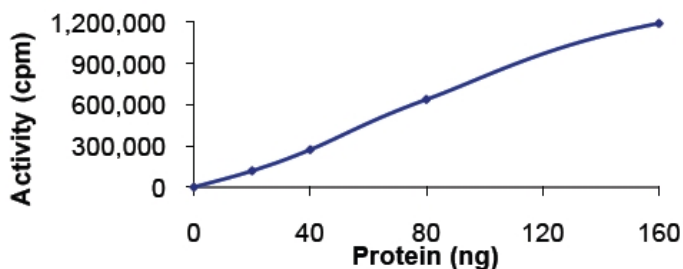
Store product at  $-70^{\circ}\text{C}$ . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

#### Stability:

Unopened vial at  $-70^{\circ}\text{C}$ , for 1 year after delivery.

**Specific Activity:**

The specific activity was determined to be 310 nmol /min/mg as per Activity Assay Protocol.

**Activity Assay Protocol:**

Assay activity of the kinase in a 25  $\mu$ L reaction consisting of 5  $\mu$ L of 5 X Kinase Assay Buffer, 5  $\mu$ L of 1 mg/ml the Substrate Solution, 5  $\mu$ L of 0.5mM AMP solution, 5  $\mu$ L of diluted kinase and 5  $\mu$ L of 250  $\mu$ M ATP solution containing [ $\gamma$ - $^{32}$ P] ATP (0.167  $\mu$ Ci/ $\mu$ L). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20  $\mu$ 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 H<sub>2</sub>O to a final concentration of 1mg/ml.

**5 X Kinase Assay Buffer:**

25mM MOPS, pH 7.2, 12.5mM  $\beta$ -glycerol-phosphate, 25mM MgCl<sub>2</sub>, 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.Foretz, M. et al: Short-term overexpression of a constitutively active form of AMP-activated protein kinase in the liver leads to mild hypoglycemia and fatty liver. *Diabetes*, 2005; 54 (5);1331-1339.

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