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1-866-9 KINASE (54-6273)

Catalogue #	Aliquot Size
P56-10H-05	5 µg
P56-10H-10	10 µg
P56-10H-20	20 µg

AMPK (A1/B1/G3), Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # P56-10H
Lot # E299-2

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.

Storage and Stability

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.

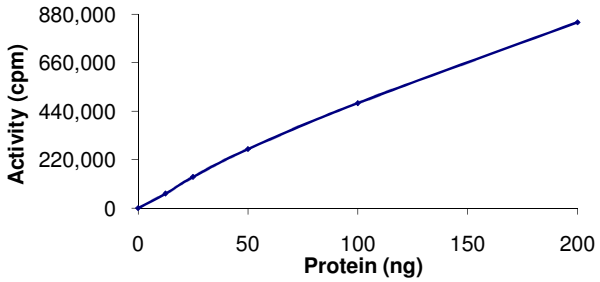
Scientific Background

AMPK (A1/B1/G3) is a member of the AMPK family which are heterotrimeric proteins consisting of an alpha catalytic subunit, and non-catalytic beta and gamma 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 beta-methylglutaryl-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).

References

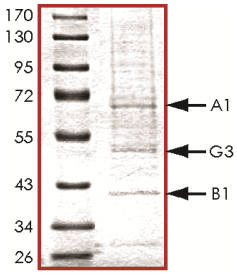
1. Viollet, B. et al: Physiological role of AMP-activated protein kinase (AMPK): insights from knockout mouse models. Biochem. Soc. Trans. 2003; 31; 216–219.
2. Cheung, P. C. F. et al: Characterization of AMP-activated protein kinase gamma-subunit isoforms and their role in AMP binding. Biochem. J. 346: 659-669, 2000.

Specific Activity



The specific activity of AMPK was determined to be **212 nmol /min/mg** as per activity assay protocol.

Purity



The purity of AMPK was determined to be **>70%** by densitometry, approx. MW **~68kDa (A1), ~38kDa (B1),** and **~51kDa (G3)**.

AMPK (A1/B1/G3), Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	P56-10H
Specific Activity	212 nmol/min/mg
Lot Specific Number	E299-2
Purity	>70%
Concentration	0.1 µg/µl
Stability	1yr At –70°C from date of shipment
Storage & Shipping	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. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: P56-10H)

Active AMPK (0.1µg/µl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active AMPK for optimal results).

Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/µl BSA.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 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.

[^{33}P]-ATP Assay Cocktail

Prepare 250µM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [^{33P}]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at –20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at –20°C.

Substrate (Catalog #: S07-58)

SAMS synthetic peptide substrate (HMRSAMSGLHLVKRR) diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active AMPK, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.

Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:

- **Component 1.** 10µl of diluted Active AMPK (Catalog # P56-10H)
- **Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog # S07-58)
- **Component 3.** 5µl of 0.5mM AMP solution (Catalog # A46-09)

Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H_2O.

Step 5. Initiate the reaction by the addition of 5 µl [^{33}P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.

Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20 µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H_2O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [P^{33}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [^{33}P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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