

AMPK (A1/B1/G3), Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # P56-10H

Lot # Q114-1

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 tags. 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, 0.2mM 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.

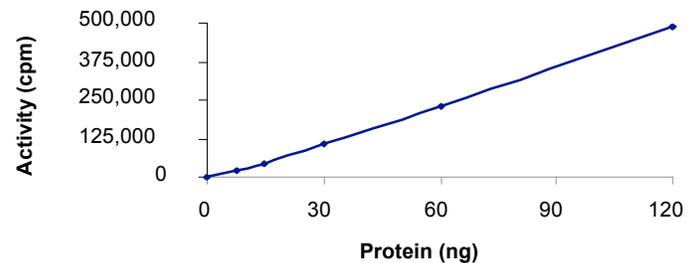
Scientific Background

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

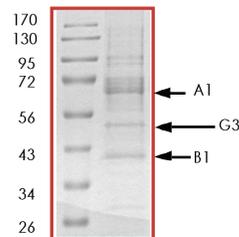
1. Viollet, B. et al: Physiological role of AMP-activated protein kinase (AMPK): insights from knockout mouse models. *Biochem. Soc. Trans.* 2003; 31; 216-219.
2. Cheung, P. C. F. et al: Characterization of AMP-activated protein kinase gamma-subunit isoforms and their role in AMP binding. *Biochem. J.*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

Specific Activity



The specific activity of AMPK was determined to be **185 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)**.

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Full-length recombinant protein expressed in Sf9 cells

Catalog Number

P56-10H

Specific Activity

185 nmol/min/mg

Lot Specific Number

Q114-1

Purity

>70%

Concentration

0.1 $\mu\text{g}/\mu\text{l}$

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 (0.1 µg/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 MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250µM [³³P]-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 (HMRSAMSGHLVKRR) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-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 H₂O.
- Step 5.** Initiate the reaction by the addition of 5 µl [³³P]-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 H₂O) 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 [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [³³P]-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 ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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