570-5600 Parkwood Way
Richmond, BC V6V2M2
CANADA
Tel: 1-(604)-232-4600
Fax: 1-(604)-232-4601
orders@signalchem.com

AMPK (A1/B1/G1), Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # P47-10H
Lot # L290-2

Product Description

Recombinant full-length human AMPK (combination of A1/B1/G1 subunits) was expressed by baculovirus in Sf9 insect cells using C-terminal His tags. The gene accession numbers for the three subunits (A1/B1/G1) are NM_006251, NM_006253, and NM_002733.

Gene Aliases

Subunit A1: PRKAA1, MGC33776, MGC57364
Subunit B1: PRKAB1, AMPK, HAMPKb, MGC17785
Subunit G1: PRKAG1, AMPKG, MGC8666

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.

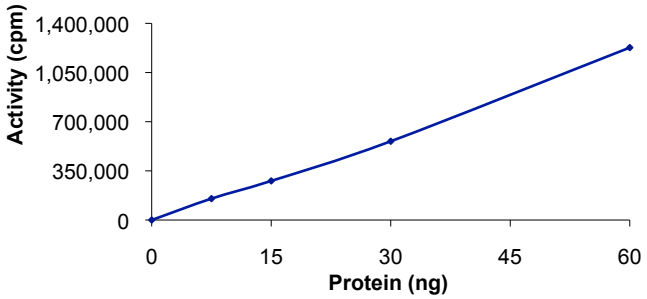
Scientific Background

AMPK is a heterotrimer protein kinase consisting of a α catalytic subunit, and non-catalytic β and γ subunits. AMPK is an important energy-sensing enzyme that monitors cellular energy status (1). In response to cellular metabolic stresses, AMPK is activated and phosphorylates and inactivates acetyl-CoA carboxylase (ACC) and beta-hydroxy beta-methylglutaryl-CoA reductase (HMGCR), key enzymes involved in regulating biosynthesis of fatty acid and cholesterol (2).

References

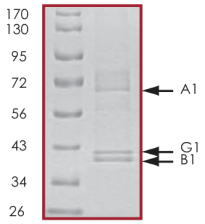
1. Minokoshi, Y. et al:AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. Nature 428: 569-574, 2004.
2. Hardie, D G. et al: The AMP-activated protein kinase--fuel gauge of the mammalian cell? Eur J Biochem. 1997 Jun 1;246(2):259-73.

Specific Activity



The specific activity of AMPK was determined to be **816 nmol /min/mg** as per activity assay protocol.

Purity



The purity of AMPK was determined to be **>95%** by densitometry, approx. MW **~68kDa (A1)**, **~38kDa (B1)**, and **~40kDa (G1)**.

AMPK (A1/B1/G1), Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	P47-10H
Specific Activity	816 nmol/min/mg
Specific Lot Number	L290-2
Purity	>95%
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 #: P47-10H)

Active AMPK (0.1μg/μl) diluted with Kinase Dilution Buffer VII (Catalog #: K27-09) 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 VII (Catalog #: K27-09)

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

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

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM $MgCl_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{32}P]-ATP Assay Cocktail

Prepare 250μM [^{32}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 [^{32}P]-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)

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

Assay Protocol

Step 1. Thaw [^{32}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 #P47-10H)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate
- **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 [^{32}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^{32}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μl [^{32}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 ^{32}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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