

Catalogue #	Aliquot Size
P54-10H-05	5 µg
P54-10H-10	10 µg
P54-10H-20	20 µg

## AMPK (A2/B2/G2), Active

Full-length recombinant protein expressed in Sf9 cells

**Catalog # P54-10H**

Lot # N029-2

### Product Description

Recombinant full-length human AMPK (combination of A2/B2/G2 subunits) was expressed by baculovirus in Sf9 insect cells using a C-terminal His tag. The gene accession numbers for the three subunits (A2/B2/G2) are [NM\\_006252](#), [NM\\_005399](#), and [NM\\_001040633](#).

### Gene Aliases

Subunit A2: PRKAA2, AMPK, AMPK2, PRKAA

Subunit B2: PRKAB2, MGC61468

Subunit G2: PRKAG2, AAKG, CMH6, WPWS, AAKG2, H91620p

### 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  $-70^{\circ}\text{C}$ . 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.

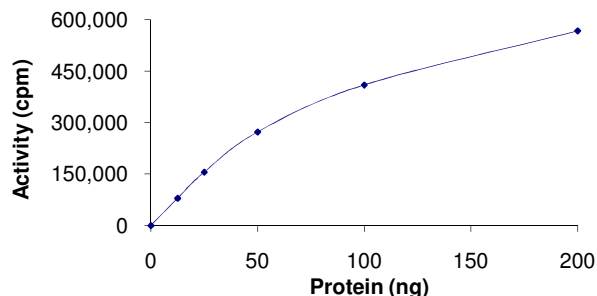
### Scientific Background

AMP-activated protein kinase (AMPK) exhibits a key role as a master regulator of cellular energy homeostasis (1). AMPK exists as a heterotrimeric complex composed of a catalytic  $\alpha$  subunit and regulatory  $\beta$  and  $\gamma$  subunits. Binding of AMP to the  $\gamma$  subunit allosterically activates the complex. AMPK is activated in response to stresses that deplete cellular ATP (low glucose, hypoxia and ischemia) (2) and via signaling pathways in response to adiponectin, leptin and CAMKK $\beta$ .

### References

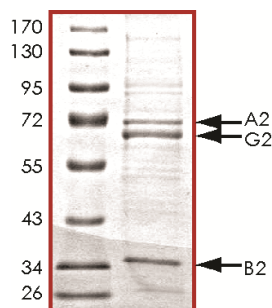
1. Hardie, G.D. The AMP-activated protein kinase pathway – new players upstream and downstream. *J. Cell Sci.* 2004;117: 5479–5487.
2. Kahn, B.B. et al. AMP-activated protein kinase: Ancient energy gauge provides clues to modern understanding of metabolism. *Cell Metab.* 2005; 1, 15–25.

### Specific Activity



The specific activity of AMPK was determined to be **310 nmol /min/mg** as per activity assay protocol.

### Purity



The purity of AMPK was determined to be **>75%** by densitometry, approx. MW **~69kDa (A2)**, **~36kDa (B2)**, and **~65kDa (G2)**.

## AMPK (A2/B2/G2), Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	P54-10H
Specific Activity	310 nmol/min/mg
Specific Lot Number	N029-2
Purity	>75%
Concentration	0.1 µg/µl
Stability	1yr At $-70^{\circ}\text{C}$ from date of shipment
Storage & Shipping	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. Product shipped on dry ice.

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# Activity Assay Protocol

## Reaction Components

### Active Kinase (Catalog #: P54-10H)

Active AMPK (A2/B2/G2) (0.1µg/µl) diluted with Kinase Dilution Buffer (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 (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/µl BSA solution.

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

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM MgCl<sub>2</sub>, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>33</sup>P]-ATP Assay Cocktail

Prepare 250µM [<sup>33</sup>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 [<sup>33</sup>P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer (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 (Catalog #: K01-09). Store 200µl aliquots at -20°C.

### Substrate (Catalog #: S07-58)

SAMStide synthetic peptide substrate (HMRSAMSGHLHLVKRR) diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

## Assay Protocol

- Step 1.** Thaw [<sup>33</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active AMPK (A2/B2/G2), Kinase Assay Buffer, Substrate and Enzyme 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 #P54-10H)
  - 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<sub>2</sub>O.
- Step 5.** Initiate the reaction by the addition of 5 µl [<sup>33</sup>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<sub>2</sub>O) 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 [<sup>33</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

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

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