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# Product Information

**AMPK (A1/B1/G3), Active, His-tagged, human**
**Precisio™ Kinase**
**recombinant, expressed in *Sf*9 cells**

Catalog Number **A4486**
Lot Number 109K0813
Storage Temperature –70 °C

Synonyms:
A1: PRKAA1, MGC33776, MGC57364
B1: PRKAB1, HAMPKb, MGC17785
G3: PRKAG3

**Product Description**
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 β-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]

This recombinant product was expressed by baculovirus in *Sf*9 insect cells using an N-terminal His-tag. The gene accession numbers are NM 006251, NM 006253 and NM 017431. It is supplied in 50 mM sodium phosphate, pH 7.0, with 300 mM NaCl, 150 mM imidazole, 0.2 mM DTT, 0.1 mM PMSF, and 25% glycerol.

Molecular mass:
A1 ~68 kDa
B1 ~38 kDa
G3 ~51 kDa

Purity: ≥70% (SDS-PAGE, see Figure 1)

Specific Activity: 157–213 nmole/min/mg (see Figure 2)

**Precautions and Disclaimer**
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

**Storage/Stability**
The product ships on dry ice and storage at –70 °C is recommended. After opening, aliquot into smaller quantities and store at –70 °C. Avoid repeated handling and multiple freeze/thaw cycles.

**Figure 1.**
SDS-PAGE Gel of Lot Number 109K0813
>70% (densitometry)

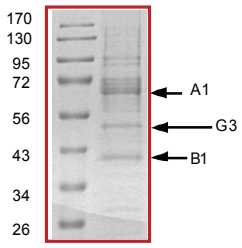
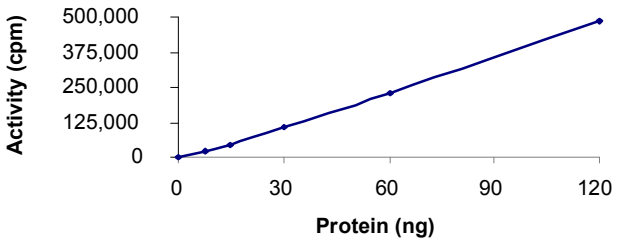



**Figure 2.**
Specific Activity of Lot Number 109K0813:
185 nmole/min/mg




**Procedure**
Preparation Instructions
Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM $MgCl_2$, 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with a 50 ng/µl BSA solution.

Kinase Solution – Dilute the Active AMPK (A1/B1/G3) (0.1 μg/μl) with Kinase Dilution Buffer to the desired concentration.
Note: The lot-specific specific activity plot may be used as a guideline (see Figure 2). It is recommended that the researcher perform a serial dilution of Active AMPK (A1/B1/G3) kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200 μl aliquots at –20 °C.

γ-$^{32}$P-ATP Assay Cocktail (250 μM) – Combine 5.75 ml of Kinase Assay Buffer, 150 μl of 10 mM ATP Stock Solution, 100 μl of γ-$^{32}$P-ATP (1 mCi/100 μl). Store in 1 ml aliquots at –20 °C.

Substrate Solution – Dissolve the synthetic peptide substrate (HMRSAMSGLHLVKRR) in water at a final concentration of 1 mg/ml.

1% phosphoric acid solution – Dilute 10 ml of concentrated phosphoric acid to a final volume of 1 L with water.

Kinase Assay
This assay involves the use of the $^{32}$P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the Active AMPK (A1/B1/G3), Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-$^{32}$P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 μl:
   10 μl of Kinase Solution
   5 μl of Substrate Solution
   5 μl of 0.5 mM AMP solution
3. Set up a blank control as outlined in step 2, substituting 5 μl of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 μl of the γ-$^{32}$P-ATP Assay Cocktail, bringing the final reaction volume to 25 μl. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20 μl of the reaction mixture onto an individually precut strip of phosphocellulose P81 paper.
6. Air dry the precut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of ~10 minutes each.
7. Set up a radioactive control to measure the total γ-$^{32}$P-ATP counts introduced into the reaction. Spot 5 μl of the γ-$^{32}$P-ATP Assay Cocktail on a precut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

Calculations:
1. Specific Radioactivity (SR) of ATP (cpm/nmole)

$$SR = \frac{\text{cpm of 5 μl of γ-}^{32}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

   cpm – value from control (step 7)
   nmole – 1.25 nmole (5 μl of 250 μM ATP Assay Cocktail)

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta\text{cpm} \times (25/20)}{SR \times E \times T}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)
Δcpm = cpm of the sample – cpm of the blank (step 3)
25 = total reaction volume
20 = spot volume
T = reaction time (minutes)
E = amount of enzyme (mg)

**References**

1. Viollet, B. et al., Physiological role of AMP-activated protein kinase (AMPK): insights from knockout mouse models. Biochem. Soc. Trans., **31**, 216–219 (2003).
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).

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