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Product Information

AMPK (α2/β2/γ2), active, His-tagged, human
PRECISIO® Kinase
recombinant, expressed in *Sf*9 cells

Catalog Number **SRP5004**
Storage Temperature –70 °C

Synonyms:
α2: PRKAA2, AMPK, AMPK2, PRKAA
β2: PRKAB2, MGC61468
γ2: PRKAG2, AAKG, CMH6, WPWS, AAKG2, H91620p

Product Description

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 α subunit and regulatory β and γ subunits. Binding of AMP to the γ subunit allosterically activates the complex. AMPK is activated in response to stresses that deplete cellular ATP (low glucose, hypoxia, and ischemia) and via signaling pathways in response to adiponectin, leptin, and CAMKKβ.[2]

Recombinant full-length human AMPK (combination of α2/β2/γ2 subunits) was expressed by baculovirus in *Sf*9 insect cells using C-terminal His tags. The gene accession numbers for the three subunits (α2/β2/γ2) are NM_006252, NM_005399, and NM_001040633, respectively. Recombinant protein stored in 50 mM sodium phosphate, pH 7.0, 300 mM NaCl, 150 mM imidazole, 0.1 mM PMSF, 0.25 mM DTT, and 25% glycerol.

Molecular mass:
α2 ~69 kDa
β2 ~36 kDa
γ2 ~65 kDa

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 Typical Lot:
Purity: 70–95% (SDS-PAGE, densitometry)

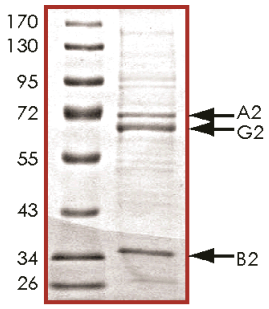
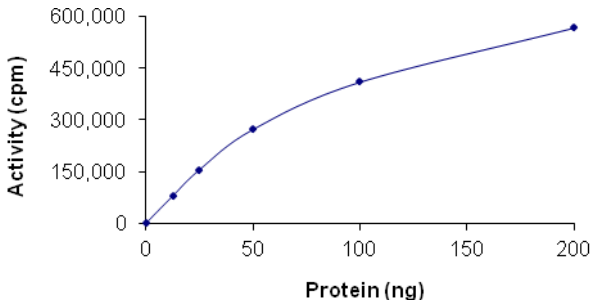


Figure 2.
Specific Activity of Typical Lot:
255–345 nmole/min/mg



Procedure

Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM $MgCl_2$, 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.

Kinase Solution – Dilute the active AMPK (α2/β2/γ2), (0.1 μg/μl) with Kinase Dilution Buffer to the desired concentration.
Note: The specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active AMPK (α2/β2/γ2) 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-^{33}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-^{33}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 ^{33}P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active AMPK (α2/β2/γ2), Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-^{33}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-^{33}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-^{33}P-ATP counts introduced into the reaction. Spot 5 μl of the γ-^{33}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)

$$SR = \frac{\text{cpm of 5 μl of γ-}^{33}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

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

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