

AMPK (A1/B1/G2), Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # P55-10H-10

Lot # Q176-2

Product Description

Recombinant full-length human AMPK (combination of A1/B1/G2 subunits) was expressed by baculovirus in Sf9 insect cells using a C-terminal His tag. The gene accession number for the three subunits (A1/B1/G2) are [NM_006251](#), [NM_006253](#), and [NM_001040633](#).

Gene Aliases

Subunit A1: PRKAA1, MGC33776, MGC57364

Subunit B1: PRKAB1, AMPK, HAMPKb, MGC17785

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

Concentration

0.1 µg/µl

Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 7.0, 300mM NaCl, 150mM imidazole, 0.1mM PMSF, 0.25mM DTT, 25% glycerol.

Storage, Shipping 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. Stability is 1yr at -70°C from date of shipment. Product shipped on dry ice.

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 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) (2) and via signaling pathways in response to adiponectin, leptin and CAMKK β .

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.

Purity

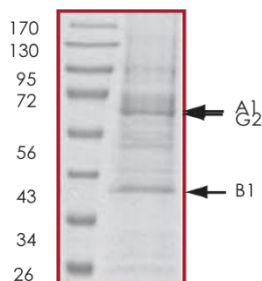
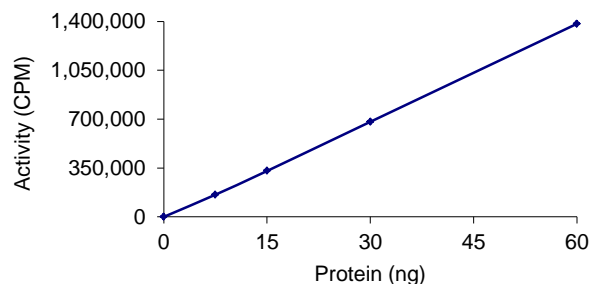


Figure 1. SDS-PAGE gel image

The purity of AMPK was determined to be **>70%** by densitometry, approx. MW **~68kDa (A1)**, **~38kDa (B1)**, and **~65kDa (G2)**.

Specific Activity

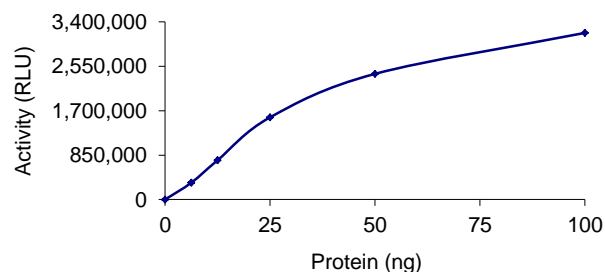
Figure 2. Radiometric Assay Data



The specific activity of AMPK was determined to be **980 nmol /min/mg** as per activity assay protocol.

(For Radiometric Assay Protocol on this product please see pg. 2)

Figure 3. ADP-Glo™ Assay Data



The specific activity of AMPK was determined to be **1050 nmol /min/mg** as per activity assay protocol.

(For ADP-Glo™ Assay Protocol on this product please see pg. 3)

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: P55-10H)

Active AMPK (0.1 µg/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 (Catalog #: K23-09)

Kinase Assay Buffer (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 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)

SAMStide synthetic peptide substrate (HMRSAMSGLHLVKRR) diluted in distilled H₂O to a final concentration of 1 mg/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 #P55-10H)
 - 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 5µl [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 20µl 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 (cpm) 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)]

ADP-Glo™ Activity Assay Protocol

Reaction Components

AMPK (A1/B1/G2) Kinase Enzyme System (Promega, Catalog #:V4012)

AMPK (A1/B1/G2), Active, 10µg (0.1µg/µl)
SAMStide, 1ml (1mg/ml)
Reaction Buffer A (5X), 1.5ml
DTT (0.1M), 25µl
AMP Solution (0.5mM), 1 ml

Reaction Buffer A (5X)

200mM Tris-HCl, pH 7.5, 100mM MgCl₂ and 0.5 mg/ml BSA.

ADP-Glo™ Kinase Assay Kit (Promega, Catalog #: V9101)

Ultra Pure ATP solution, 10 mM (0.5ml)
ADP solution, 10 mM (0.5ml)
ADP-Glo™ Reagent (5ml)
Kinase Detection Buffer (10ml)
Kinase Detection Substrate (Lyophilized)

Assay Protocol

The AMPK (A1/B1/G2) assay is performed using the AMPK (A1/B1/G2) Kinase Enzyme System (Promega; Catalog #: V4012) and ADP-Glo™ Kinase Assay kit (Promega; Catalog #: V9101). The AMPK (A1/B1/G2) reaction utilizes ATP and generates ADP. Then the ADP-Glo™ Reagent is added to simultaneously terminate the kinase reaction and deplete the remaining ATP. Finally, the Kinase Detection Reagent is added to convert ADP to ATP and the newly synthesized ATP is converted to light using the luciferase/luciferin reaction. For more detailed protocol regarding the ADP-Glo™ Kinase Assay, see the technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html.

- Step 1.** Thaw the ADP-Glo™ Reagents at ambient temperature. Then prepare Kinase Detection Reagent by mixing Kinase Detection Buffer with the Lyophilized Kinase Detection Substrate. Set aside.
- Step 2.** Thaw the components of AMPK (A1/B1/G2) Enzyme System, ADP and ATP on ice.
- Step 3.** Prepare 1ml of 3X Buffer by combining 600µl Reaction Buffer A, 1.5µl DTT and 398.5µl of dH₂O.
- Step 4.** Prepare 1ml of 250µM ATP Assay Solution by adding 25µl ATP solution (10mM) to 333µl of 3X Buffer and 642µl of dH₂O.
- Step 5.** Prepare diluted AMPK (A1/B1/G2) in 1X Buffer (diluted from 3X buffer) 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 (A1/B1/G2) for optimal results).
- Step 6.** In a white 96-well plate (Corning Cat # 3912), add the following reaction components bringing the initial reaction volume up to 20µl:

Component 1.	5µl of diluted Active AMPK(A1/B1/G2)
Component 2.	5µl of 1mg/ml stock solution of substrate
Component 3.	5µl of 0.5mM AMP solution
Component 4.	5µl of 3X Buffer
- Step 7.** Set up the blank control as outlined in step 6, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
- Step 8.** At the same time as the AMPK (A1/B1/G2) kinase reaction, set up an ATP to ADP conversion curve at 50µM ATP/ADP range as described in the ADP-Glo™ Kinase Assay technical Manual #TM313.
- Step 9.** Initiate the AMPK (A1/B1/G2) reactions by the addition of 5µl of 250 µM ATP Assay Solution thereby bringing the final volume up to 25µl. Shake the plate and incubate the reaction mixture at 30°C for 15 minutes.
- Step 10.** Terminate the reaction and deplete the remaining ATP by adding 25µl of ADP-Glo™ Reagent. Shake the 96-well plate and then incubate the reaction mixture for another 40 minute at ambient temperature.
- Step 11.** Add 50µl of the Kinase Detection Reagent, shake the plate and then incubate the reaction mixture for another 30 minute at ambient temperature.
- Step 12.** Read the 96-well reaction plate using the Kinase-Glo™ Luminescence Protocol on a GloMax® plate reader (Promega; Cat# E7031).
- Step 13.** Using the conversion curve, determine the amount of ADP produced (nmol) in the presence (step 6) and absence of substrate (Step 7) and calculate the kinase specific activity as outlined below. For a detailed protocol of how to determine nmols from RLUs, see Kinase Enzyme Systems Protocol at: <http://www.promega.com/KESProtocol>

Kinase Specific Activity (SA) (nmol/min/mg)

(ADP (step 6) – ADP (Step 7)) in nmol) / (Reaction time in min)*(Enzyme amount in mg)