SignalChem
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1-866-9 KINASE (54-6273)

AMPK (A2/B1/G1), Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # P48-10H-10
Lot # Q260-1

Product Description

Recombinant full-length human AMPK (combination of A2/B1/G1 subunits) was expressed by baculovirus in Sf9 insect cells using a C-terminal His tag. The gene accession numbers for the three subunits (A2/B1/G1) are NM_006252, NM_006253, and NM_002733

Gene Aliases

Subunits A2: PRKAA2, AMPK, AMPK2, PRKAA
Subunit B1: PRKAB1, AMPK, HAMPKb, MGC17785
Subunit G1: PRKAG1, AMPKG, MGC8666

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

AMPK (A2/B1/G1) plays a key role in insulin signaling pathway and is a major therapeutic target for the treatment of diabetes (1). AMPK is viewed as a fuel sensor for glucose and lipid metabolism by modulating the activity of the autonomous nervous system *in vivo*. Short-term overexpression of a constitutively active form of AMPK in the liver leads to mild hypoglycemia and fatty liver due to increased fatty acid utilization (2).

References

1. Viollet, B. et al: Physiological role of AMP-activated protein kinase (AMPK): insights from knockout mouse models. Biochem. Soc. Trans. 2003; 31; 216–219.
2. Foretz, M. et al: Short-term overexpression of a constitutively active form of AMP-activated protein kinase in the liver leads to mild hypoglycemia and fatty liver. Diabetes, 2005; 54 (5);1331-1339.

Purity

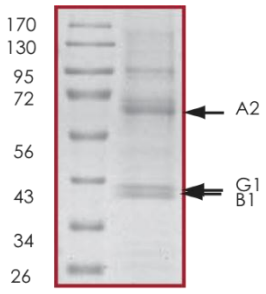
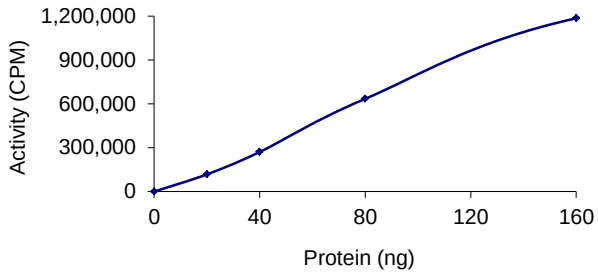


Figure 1. SDS-PAGE gel image

The purity of AMPK was determined to be **>75%** by densitometry, approx. MW **~69kDa (A2), ~38kDa (B1),** and **~40kDa (G1)**.

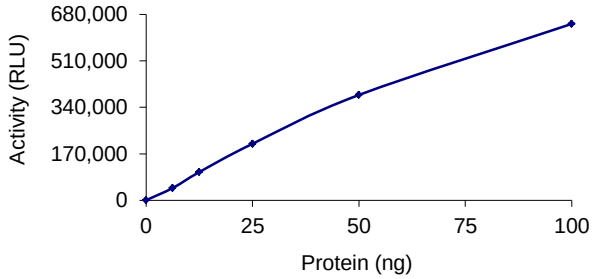
Specific Activity

Figure 2. Radiometric Assay Data



The specific activity of AMPK was determined to be **310 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 **160 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 #: P48-10H)

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 $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250μM [^{33}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 [^{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 H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{33}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 #P48-10H)
- **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_2O.

Step 5. Initiate the reaction by the addition of 5μl [^{33}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 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 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 (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 [P^{33}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μl [^{33}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 ^{33}P-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 (A2/B1/G1) Kinase Enzyme System (Promega, Catalog #:V4014)

AMPK (A2/B1/G1), 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

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)

Reaction Buffer A (5X)

200mM Tris-HCl, pH 7. 5, 100mM $MgCl_2$ and 0.5 mg/ml BSA.

Assay Protocol

The AMPK (A2/B1/G1) assay is performed using the AMPK (A2B1G1) Kinase Enzyme System (Promega; Catalog #: V4014) and ADP-Glo™ Kinase Assay kit (Promega; Catalog #: V9101). The AMPK (A2B1G1) 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 (A2/B1/G1) 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 dH_20.

Step 4. Prepare 1ml of 250µM ATP Assay Solution by adding 25µl ATP solution (10mM) to 333µl of 3X Buffer and 642µl of dH_20.

Step 5. Prepare diluted AMPK (A2/B1/G1) 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 (A2/B1/G1) 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(A2/B1/G1)
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 H_2O.

Step 8. At the same time as the AMPK (A2/B1/G1) 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 (A/2B/1G1) 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)