

AMPK α 2 β 1 γ 1 Kinase

5 μ g

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com

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Description: Purified recombinant full-length human AMPK kinase (combination of α 2/ β 1/ γ 1 subunits), supplied as a His tag fusion protein.

Background: AMP-activated protein kinase (AMPK) is highly conserved from yeast to plants and animals and plays a key role in the regulation of energy homeostasis (1). AMPK is a heterotrimeric complex composed of a catalytic α subunit and regulatory β and γ subunits, each of which is encoded by two or three distinct genes (α 1, 2; β 1, 2; γ 1, 2, 3) (2). The kinase is activated by an elevated AMP/ATP ratio due to cellular and environmental stress, such as heat shock, hypoxia and ischemia (1). The tumor suppressor LKB1, in association with accessory proteins STRAD and MO25, phosphorylates AMPK α at Thr172 in the activation loop and this phosphorylation is required for AMPK activation (3-5). AMPK α is also phosphorylated at Thr258 and Ser485 (for α 1; Ser491 for α 2). The upstream kinase and the biological significance of these phosphorylation events have yet to be elucidated (6). The β 1 subunit is post-translationally modified by myristoylation and multi-site phosphorylation including Ser24/25, Ser96, Ser101, Ser108 and Ser182 (6,7). Phosphorylation at Ser108 of the β 1 subunit seems to be required for the activation of AMPK enzyme, while phosphorylation at Ser24/25 and Ser182 affects AMPK localization (7). Several mutations in AMPK γ subunits have been identified, most of which are located in the putative AMP/ATP binding sites (CBS or Bateman domains). Mutations at these sites lead to reduction of AMPK activity and cause glycogen accumulation in heart or skeletal muscle (1,2). Accumulating evidence indicates

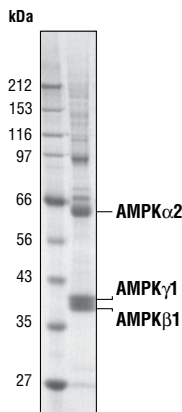


Figure 1. The purity of the AMPK α 2 β 1 γ 1 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

that AMPK not only regulates the metabolism of fatty acids and glycogen, but also modulates protein synthesis and cell growth through EF2 and TSC2/mTOR pathways, as well as blood flow via eNOS/nNOS (1).

Source/Purification: The His-Kinase fusion protein complex was produced using a baculovirus expression system with a construct expressing full-length human AMPK α 2 (Met1-Arg552) (GenBank Accession No. NM_006252), AMPK β 1 (Met1-Ile270) (GenBank Accession No. NM_006253), and AMPK γ 1 (Met1-Pro331) (GenBank Accession No. NM_002733), all with carboxy-terminal His tags. The protein was purified by Immobilized Metal Affinity Chromatography (IMAC).

Quality Control: The theoretical molecular weight of the 3 subunits of AMPK fusion protein is (α 2/ β 1/ γ 1) 69, 38, and 40 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. AMPK α 2 β 1 γ 1 kinase activity was determined using a radiometric assay [Fig.2].

Background References:

- (1) Hardie, D.G. (2004) *J. Cell Sci.* 117, 5479–5487.
- (2) Carling, D. (2004) *Trends Biochem. Sci.* 29, 18–24.
- (3) Hawley, S.A. et al. (1996) *J. Biol. Chem.* 271, 27879–27887.
- (4) Lizcano, J.M. et al. (2004) *EMBO J.* 23, 833–843.
- (5) Shaw, R. et al. (2004) *Proc. Natl. Acad. Sci. USA* 101, 3329–3335.
- (6) Woods, A. et al. (2003) *J. Biol. Chem.* 278, 28434–28442.
- (7) Warden, S.M. et al. (2001) *Biochem. J.* 354, 275–283.

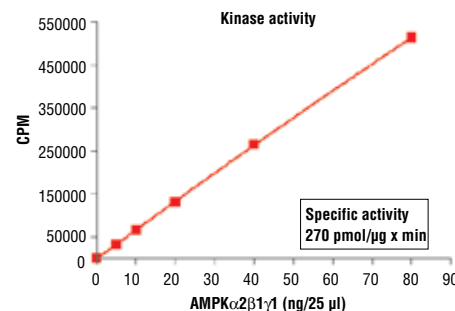


Figure 2. AMPK α 2 β 1 γ 1 kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β -glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM MgCl₂, 0.05 mM DTT, 50 μ M ATP, 0.1 mM AMP, Substrate: SAMS peptide, 200 ng/ μ l, and variable amounts of recombinant AMPK α 2 β 1 γ 1.

Protocol for AMPK α 2 β 1 γ 1 Kinase Assay

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

1. Kinase Buffer (10X)

50 mM MOPS, pH 7.2
25 mM β -glycerophosphate
10 mM EGTA
4 mM EDTA
50 mM MgCl₂
0.5 mM DTT

2. ATP (10 mM) #9804

3. AMP (0.5 mM)

4. ³²P- γ ATP

5. SAMS peptide (HMRSAMSGLVKRR, 1.0 μ g/ μ l)

B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 μ M ATP.
2. Dilute [³²p] ATP to 0.16 μ Ci/ μ l [³²p] ATP with 250 μ M ATP solution.
3. Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
4. Dilute AMPK α 2 β 1 γ 1 kinase protein (100 ng/ μ l concentration) to 10 ng/ μ l with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 μ l diluted AMPK α 2 β 1 γ 1 kinase solution, 5 μ l SAMS peptide (1.0 μ g/ μ l), 5 μ l 0.5 mM AMP, and 5 μ l 0.16 μ Ci/ μ l [³²p] ATP solution.

Final Assay Conditions

5 mM MOPS, pH 7.2
2.5 mM β -glycerophosphate
1 mM EGTA
0.4 mM EDTA
5 mM MgCl₂
0.05 mM DTT
50 ng/ μ l ATP
0.1 mM AMP
200 ng/ μ l SAMS peptide

6. After 15 minutes terminate reaction by spotting 20 μ l of the reaction mixture onto phosphocellulose P81 paper.
7. Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
8. Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
9. Count samples in a scintillation counter.

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