ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

AMP Activated Kinase- β1/2 (AMPK- β1/2) Antibodies

Cat. # AMPKB11-A	Rabbit Anti-Human AMPK-βı IgG (Aff pure)	**SIZE**: 100 ug
Cat. # AMPKB11-P	Human AMPK-βı Control/blocking peptide	**SIZE**: 100 ug

In cells, excess of metabolic fuel is converted into fatty acids in cytosol and oxidized later in mitochondria to generate ATP and acetyl-CoA. In fatty acid synthesis, catalytic formation of malonyl-CoA (precursor for long-chain fatty acyl-CoA, LCFA-CoA) from acetyl-CoA by **Acetyl-CoA carboxylase** (**ACC-1**) is the rate limiting step. The translocation of LCFA-CoA from cytosol to mitochondria, catalyzed by two **carnitine palmitoyl transferases (CPT-1** & **CPT-2**) and regulated by **ACC-2**, is the rate limiting step of mitochondrial fatty acid □-oxidation. Activities of ACC-1, ACC-2 and other key proteins of carbohydrate and fat metabolism are regulated by their phosphorylation by 5'-AMP-activated protein kinase **(AMPK)**. AMPK switches-off biosynthetic processes when ATP levels are depleted and AMP rises in response to fuel deficiency and treatments like heat shock, ischaemia and exercise. AMPK also regulates cholesterol biosynthesis via phosphorylation and inactivation of hormone-sensitive lipase and hydroxymethylglutaryl-CoA reductase. It also appears to act as a metabolic stress-sensing protein kinase switching off biosynthetic pathway when ATP levels are depleted and when 5-AMP rises in response to fuel limitation and or hypoxia

AMPK is a heterotrimer of a catalytic subunit □ (~63 kDa), and two non-catalytic regulatory subunits, □ (~40 kDa) and □ (~38 kDa). These subunits exist in multiple isoforms (□$_1$, □$_2$, □$_1$, □$_2$, □$_1$ and □$_2$). Coexpression of all three subunit is required for kinase activity. AMPK-beta acts as a scaffold vial which trimeric complex of AMPK assembles, with the □-subunit binding to its KIS domain and the g-subunit binding to its KIS domain and the □-subunit binding to its ACS domain. **AMPK-beta 1** (mouse/rat/human 270-aa, ~38 kDa, chromosome 12q24.1) and **AMPK Beta-2** (mouse/rat, 271-aa, human 270-aa, ~34 kDa) share ~70% identity. Both isoform contribute equally to the AMPK activity. AMPK-□1 is highly expressed in liver and brain and low levels in kidney and skeletal muscle. AMPK-□2 is most abundant in skeletal muscle with low levels in kidney, liver, and lung.

Source of Antigen and Antibodies

Antigen	15-aa peptide of human AMPK-**β1/2** (gene accession # O43741 PRKAB2; **Designated (AMPKB11-P) control peptide** conjugated to KLH, Epitope location ~ C-terminus
Ab Host/type	Rabbit, polyclonal, purified over antigen-agarose column (**cat #AMPKB11-A**) purified over antigen-agarose column
2-ab	**Goat Anti-rabbit IgG-HRP** cat # 20320 (AP, biotin, FITC conjugates also available)
-ve	# 20009-1, Rabbit (non-immune) IgG, purified, suitable for ELISA, Western, IHC as –ve control

Form & Storage of Antibodies/Peptide Control

Affinity pure IgG
100 ug/100ul solution lyophilized powder
Supplied in **Buffer**: PBS+0.1% BSA
Reconstitute powder in PBS at 1mg/ml

Control/blocking peptide
100 ug/100 ul solution lyophilized powder
Supplied in Buffer: PBS pH 7.5,
Reconstitute powder in PBS at 1 mg/ml.

Storage

Short-term: unopened, undiluted liquid vials at -20OC and powder at 4oC or -20oC..

Long-term: at –20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at –20oC or below.
Shipping: 4oC for solutions and room temp for powder.

Recommended Usage

Western Blotting (1-10 ug/ml for affinity pure using Chemiluminescence technique).

ELISA (1:10K-1:100K; using 50-100 ng of control peptide/well).

Histochemistry & Immunofluorescence: not tested. We recommend the use of affinity pure antibody at 2-20 ug/ml.

Specificity & Cross-reactivity

The human AMPKB11-P peptide is 100% conserved in human, mouse and rat AMPK-□ıand AMPK-□□. The AMPKB11-P is 93% conserved in Drosophila proteins CG8057 (341-aa), GH2668p (220-aa) and 100% conserved in C. elegans F55F3.1.p protein (269-aa), Y47D3A.15.p protein (274-aa). Anti-AMPKB11 recognize both □1 and □2 isoform of AMPK. No significant sequence homology of AMPKB11-P is seen with AMPK□ǫr□isoforms. Antibody reactivity in various species is not known. The AMPKB11-P, Control peptide, because of its low mol. Wt (<3 kDa), is not suitable for Western. It should be used for ELISA or antibody blocking experiments (use 5-10 ug control peptide per 1 ug of aff pure IgG or 1 ul antiserum) to confirm antibody specificity (see detailed protocol at: web site).

General References: (1) Thornton C et al (1998) JBC 12443-12450; Stapelton D et al (1997) FEBS Lett. 409, 452-456; Woods A et al (1996) JBC 271, 10282-10290; Winder WW et al (1999) Am. J. Physiol. 277, E1-E10 (review)

**This product is for In vitro research use only.*

Related materials available from ADI

Antibodies: ACC-1, ACC2, CPT-1 and CPT2, AMPK1 & 2.

AMPKB11-A-P 71208S

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