ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

AMP Activated Kinase-α_1 (AMPK-α_1) Antibodies

Cat. # AMPKA11-S	Rabbit Anti-Mouse AMPK-α_1 antiserum	**SIZE**: 100 ul
Cat. # AMPKA11-A	Rabbit Anti-Mouse AMPK- α_1 IgG #1 (Aff pure)	**SIZE**: 100 ug
Cat. # AMPKA11-P	Mouse AMPK-α_1 Control/blocking peptide#1	**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.

AMPK is a heterotrimer of a catalytic subunit □ (~63 kDa), and two non-catalytic 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. The expression of □$_2$ subunit (552aa) is most abundant in skeletal muscle with lower levels in liver, heart, lung and kidney. In contrast, □$_1$ subunit (548aa) is expressed at very low levels in all the tissues. **AMPK-□$_1$** or **AAK1** is more AMP dependent than **AMPK-□$_2$**. The aa sequences of □$_1$ and □$_2$, in their catalytic core and C-terminal tails are ~90 % and 60 %, respectively, identical.

Source of Antigen and Antibodies

Antigen	20-aa peptide of mouse AMPK- α_1 (gene accession # P54645, AAK1 refs 1); **Designated (AMPK11-P) control peptide /blocking peptide**) conjugated to KLH. Epitope location ~ C-terminus.
Ab Host/type	Rabbit, Polyclonal unpurified antiserum (#**AMPK11**-S) and IgG, purified over antigen-agarose (Cat # **AMPK11**-A)
2-Ab	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available)
-ve control	# 20009-1, Rabbit (non-immune) IgG, purified, suitable for ELISA, Western, IHC as –ve control

Form & Storage of Antibodies/Peptide Control

Antiserum (unpurified)
100ul solution lyophilized powder
Supplied in Buffer: 0.05% azide
Reconstitute powder in 100 ul PBS

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 (1:1K-5K for neat serum and 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

Mouse AMPKA11-P peptide is 95% conserved in rat and human AMPK-α_1. No significant sequence homology of AMPKA11-P is seen with AMPK–α_2 or other proteins. Antibody reactivity in various species is not known. The AMPKA11-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) Stapleton et al. (1996) J. Biol. Chem. 271, 611; Stapelton D et al (1994) JBC 269, 29343; 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.

AMPK11-S-A-P 71208S

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