ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

AMP Activated Kinase-α2 (AMPK-α2) Antibodies

Cat. # AMPKA21-S	Rabbit Anti-Rat AMPK-α2 antiserum #1	**SIZE**: 100 ul
Cat. # AMPKA21-A	Rabbit Anti-Rat AMPK-α2 IgG #1 (Aff pure)	**SIZE**: 100 ug
Cat. # AMPKA21-P	Rat AMPK-α2 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.

AMPK is a heterotrimer of a catalytic subunit α (~63 kDa), and two non-catalytic subunits, β (~40 kDa) and γ (~38 kDa). These subunits exist in multiple isoforms (α_1, α_2, β_1, β_2, γ_1 and γ_2). Coexpression of all three subunit is required for kinase activity. The expression of α_2 subunit (552aa) is most abundant in skeletal muscle with lower levels in liver, heart, lung and kidney. In contrast, α_1 subunit (548aa) is expressed at very low levels in all the tissues. **AMPK-α_1** or **AAK1** is more AMP dependent than **AMPK-α_2.** The aa sequences of α_1 and α_2, in their catalytic core and C-terminal tails are ~90 % and 60 %, respectively, identical..

Source of Antigen and Antibodies

Antigen	20aa peptide of rat AMPK-a **2** (gene accession # Q09137 AAK2); **Designated (AMPKA21-P) Control/blocking peptide**) conjugated to KLH.Epitope location ~ C-terminus
Ab Host/type	Rabbit, Polyclonal unpurified antiserum (#**AMPKA21**-S) and IgG, purified over antigen-agarose (Cat # **AMPKA21**-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

The rat AMPKA21-P peptide is 85% conserved in human, pig and 90% in mouse AMPK-α2. No significant sequence homology of AMPKA21-P is seen with AMPK–α1 or other proteins. Antibody reactivity in various species is not known. The AMPKA21-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) Carlington D et al (1994) JBC 269, 11442-11448; Gao G et al (1995) BBA 1266, 73-82; Aguan K et al (1994) Gene 149, 345-350; Beri RK et al (1994) FEBS Lett. 356, 117-121; 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

AMPKA21-S-A-P 70718A

Alpha Diagnostic Intl Inc., 6203 Woodlake Center Dr, San Antonio, TX 78244, USA; **Email**: service@4adi.com
☎ (800) 786-5777; (210) 561-9515; **Fax**: (210) 561-9544; **Web Site**: www.4adi.com