AMPKβ1 [pS182] ABfinity™ Recombinant Rabbit Monoclonal Antibody - Purified

REF Catalog no. 700241

(See product label for lot information)

Clone/PAD: 9H26L42
Isotype: IgG
Gene ID: 5564
Protein Acc. no.: Q9Y478
Qty: 100 µg
Volume: 200 µl
Concentration: 0.5 mg/ml

Formulation
PBS + 0.09% azide

Immunogen
A peptide corresponding to amino acids 177-189 of Q9Y478.

Immunogen sequence
SELSS[pS]PPGPYHQ

Reactivity
This antibody reacts with human AMPKβ1 [pS182]. Based on sequence identity and similarity, reactivity to mouse, rat, equine, opossum, bovine, orangutan, chicken, swine, canine, carp, zebrafish, platypus, salmon, and Xenopus is expected.

Specificity
This antibody is specific for AMPKβ1 [pS182] and does not recognize non-phosphorylated AMPKβ1.

Storage
2-8°C for up to 1 mo, -20°C for long term storage. Avoid repeated freezing and thawing.

Expiration Date
Expires one year from date of receipt when stored as instructed.

Validated Applications:

	Species	Test Material	Concentration
Western Blotting	human	Jurkat	2-3 µg/ml
Immunohistochemistry	human	colon carcinoma	4-6 µg/ml
Immunofluorescence	human	HeLa	4-6 µg/ml
Flow Cytometry	human	Jurkat	0.5-1 µg/test

Background
AMP-activated protein kinase (AMPK) is a metabolic and stress-sensing kinase that regulates homeostasis, and is a key target for treating Type 2 diabetes and obesity (1-7). AMPK exists as a heterotrimeric complex comprised of a catalytic α subunit (62 kDa) and non-catalytic β and γ subunits. The β subunit has at least three isoforms, designated β1, β2 and β3. AMPK is phosphorylated by upstream kinases, including AMPK Kinase (AMPKK) and LKB1, which results in AMPK activation (8). Activated AMPK in turn regulates metabolism by phosphorylating rate-limiting enzymes such as acetyl-CoA carboxylase and beta-hydroxy beta-methylglutaryl-CoA reductase, which are required for *de novo* fatty acid biosynthesis. The phosphorylation site recognized by this antibody is S182 of the β1 isoform. Reactivity with other β isoforms has not been determined.

References

1. Miller, E.J., et al. (2008) Macrophage migration inhibitory factor stimulates AMP-activated protein kinase in the ischaemic heart. Nature 451:578-583.
2. D'Eon, T.M., et al. (2008) Estradiol and the estradiol intermediate, 2-hydroxyestradiol, activate AMP-activated protein kinase in C2C12 myotubes. Obesity 16:1284-1288.
3. McCullough, L.D., et al. (2005) Pharmacological inhibition of AMP-activated protein kinase provides neuroprotection in stroke. J. Biol. Chem. 280(21):20493-20502.
4. Jorgensen, S.B., et al. (2004) The alpha2-5'AMP-activated protein kinase is a site 2 glycogen synthase kinase in skeletal muscle and is responsive to glucose loading. Diabetes 53(12):3074-3081.
5. Shaw, R.J., et al. (2004) The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. Proc. Natl. Acad. Sci. USA 101(10):3329-3335.
6. Zhou, M.H., et al. (2004) Activation of the AMP-activated protein kinase by the anti-diabetic drug metformin in vivo. Role of mitochondrial reactive nitrogen species. J. Biol. Chem. 279(42):43940-43951.
7. Hawley, S.A., et al. (2002) The antidiabetic drug metformin activates the AMP-activated protein kinase cascade via an adenine nucleotide-independent mechanism. Diabetes 51(8):2420-2425.
8. Woods, A., et al. (2003) Identification of phosphorylation sites in AMP-activated protein kinase (AMPK) for upstream AMPK kinases and study of their roles by site-directed mutagenesis. J. Biol. Chem. 278(31):28434-28442.

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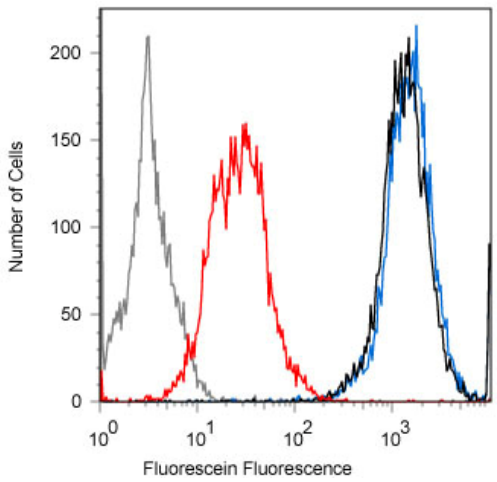
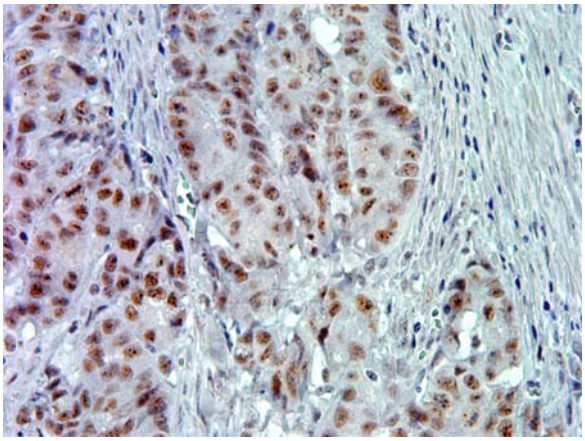


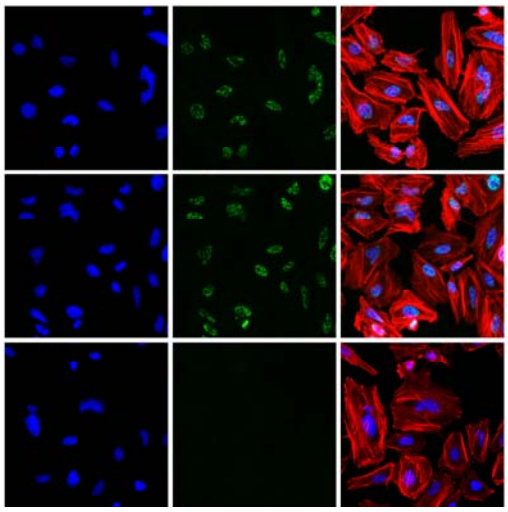
Flow cytometry of Jurkat cells labeled with rabbit anti-AMPKβ1 [pS182] (Cat. No. 700241).

Jurkat cells were fixed and permeabilized using FIX & PERM® (Cat. No. GAS004) reagents. Cells were then stained with (black trace) or without (gray trace) 0.5 µg anti-AMPKβ1 [pS182] followed by Alexa Fluor® 488 goat anti-rabbit Ig (Cat. No. A11008). Pre-incubation with the immunogenic phosphopeptide decreased the signal (red trace), whereas incubation with the non-phosphopeptide did not (blue trace).



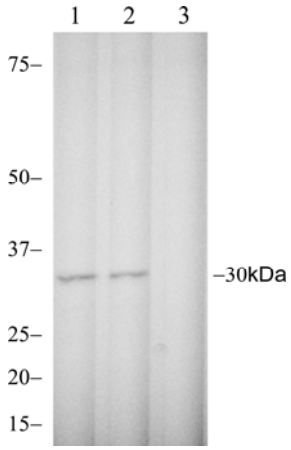
Immunohistochemistry of human colon carcimona tissue labeled with rabbit anti-AMPKβ1 [pS182] (Cat. No. 700241).

FFPE human colon carcinoma tissue was labeled with rabbit anti-AMPKβ1 [pS182] (5 µg/ml). Tissues were pretreated with EDTA and detected with SuperPicTure™ Polymer DAB (Cat. No.87-8963). Images were taken at 40x magnification. Note nuclear staining in tumor cells.



Immunocytochemistry of HeLa cells labeled with rabbit anti-AMPKβ1 [pS182] (Cat. No. 700241).

HeLa cells labeled with rabbit anti-AMPKβ1 [pS182] (2.5 µg/ml) in the absence of peptides (top panels), and presence of phosphopeptide used as immunogen (bottom panels) or non-phosphopeptide (middle panels). Alexa Fluor® 488 goat anti-rabbit (Cat. No. A11008) at 1:1000 was used as secondary antibody. Actin was stained with Alexa Fluor® 568 Phalloidin (Cat. No. A12380). Hoechst only (left), AMPKβ1 [pS182] (AF488) signal only (center), and composite image with Phalloidin (right).



Western blot of Jurkat lysates labeled with rabbit anti-AMPKβ1 [pS182] (Cat. No. 700241).

Rabbit anti-AMPKβ1 [pS182] (2.5 µg/mL) was used to label AMPKβ1 [pS182] in Jurkat lysates (lane 1). Pre-incubation with the phosphopeptide used for immunization resulted in loss of signal (lane 3) whereas pre-incubation with the non-phosphopeptide did not (lane 2).

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