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Product datasheet

AMPKα Total and Phospho T172 In-Cell ELISA Kit (IR) ab151280

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Overview

Product name AMPKa Total and Phospho T172 In-Cell ELISA Kit (IR)

Detection method IR

Precision

Intra-assay

Sample	n	Mean	SD	CV%
AMPK Total				4.7%
AMPK pT172				8.7%

Sample type Adherent cells, Suspension cells

Assay type Cell-based (quantitative)

Range 12000 cells/well - 100000 cells/well

Assay duration Multiple steps standard assay

Species reactivity **Reacts with:** Mouse, Rat, Human

Product overview ab151280 is an In-Cell ELISA (ICE) assay kit that uses quantitative immunocytochemistry to measure levels of total AMPK alpha1 subunit and phosphorylated at threonine 172 AMPK alpha1 subunit in cultured cells.

In-Cell ELISA (ICE) technology is used to perform quantitative immunocytochemistry of cultured cells with a near-infrared fluorescent dye-labeled detector antibody. The technique generates quantitative data with specificity similar to Western blotting, but with much greater quantitative precision and higher throughput due to the greater dynamic range and linearity of direct fluorescence detection and the ability to run up to 96 samples in parallel. This method rapidly fixes the cells in situ, stabilizing the in vivo levels of proteins and their post-translational modifications, and thus essentially eliminates changes during sample handling, such as preparation of protein extracts. Finally, the signal can be normalized to cell amount, measured by the provided Janus Green whole cell stain, to further increase the assay precision.

Plates are available in our ICE (In-Cell ELISA) Support Pack (**ab111542**) which can be bought seperately.

This product is designed for LI-COR® Odyssey® or Aerius® infrared imaging systems.

Notes

AMP-activated protein kinase (AMPK) is an energy sensor protein kinase that plays a key role in regulating cellular energy homeostasis. Mammalian AMPK is a heterotrimer kinase, containing a catalytic subunit (α) and two regulatory subunits (β and γ). Each subunit has different isoforms (α1, α2, β1, β2, γ1, γ2, γ3) with differential tissue expression, cellular localization and functionality. It has been hypothesized that when ADP or AMP are present at high levels, these nucleotides bind directly to the γ subunit, leading to a conformational change that allows phosphorylation of Thr172 at the α subunit. Phosphorylation of AMPK α activates the kinase which leads to downstream effects concerted to increase catabolic and suppress anabolic pathways in order to restore levels of cellular ATP and ultimately cell fate. AMPK is activated physiologically due to stresses such as low nutrients and prolonged exercise. Furthermore AMPK may be activated pharmacologically by metformin (the most widely prescribed Type 2 diabetes drug), phenformin, AICAR (acadesine/AICA riboside) and resveratrol.

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.
It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

Platform

Reagents

Properties

Storage instructions

Store at +4°C. Please refer to protocols.

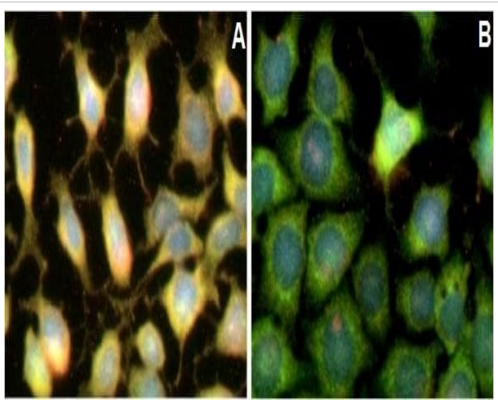
Components	1 x 96 tests
100X AMPK (total and pT172/183) Primary Antibody Cocktail	1 x 120µl
10X Blocking Solution	1 x 15ml
10X Phosphate Buffered Saline	1 x 100ml
33x Triton X-100	1 x 1.5ml
400X Tween-20	1 x 2ml
500X IRDye®-Labeled Secondary Antibody Cocktail	1 x 30µl
Antigen Retrieval Buffer	1 x 25ml
1X Janus Green Stain	1 x 17ml

Function

Catalytic subunit of AMP-activated protein kinase (AMPK), an energy sensor protein kinase that plays a key role in regulating cellular energy metabolism. In response to reduction of intracellular ATP levels, AMPK activates energy-producing pathways and inhibits energy-consuming processes: inhibits protein, carbohydrate and lipid biosynthesis, as well as cell growth and proliferation. AMPK acts via direct phosphorylation of metabolic enzymes, and by longer-term effects via phosphorylation of transcription regulators. Also acts as a regulator of cellular polarity by remodeling the actin cytoskeleton; probably by indirectly activating myosin. Regulates lipid synthesis by phosphorylating and inactivating lipid metabolic enzymes such as ACACA, ACACB, GYS1, HMGCR and LIPE; regulates fatty acid and cholesterol synthesis by phosphorylating

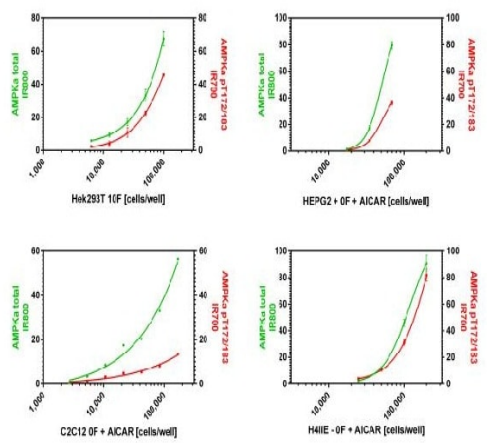
	acetyl-CoA carboxylase (ACACA and ACACB) and hormone-sensitive lipase (LIPE) enzymes, respectively. Regulates insulin-signaling and glycolysis by phosphorylating IRS1, PFKFB2 and PFKFB3. AMPK stimulates glucose uptake in muscle by increasing the translocation of the glucose transporter SLC2A4/GLUT4 to the plasma membrane, possibly by mediating phosphorylation of TBC1D4/AS160. Regulates transcription and chromatin structure by phosphorylating transcription regulators involved in energy metabolism such as CRTC2/TORC2, FOXO3, histone H2B, HDAC5, MEF2C, MLXIPL/ChREBP, EP300, HNF4A, p53/TP53, SREBF1, SREBF2 and PPARGC1A. Acts as a key regulator of glucose homeostasis in liver by phosphorylating CRTC2/TORC2, leading to CRTC2/TORC2 sequestration in the cytoplasm. In response to stress, phosphorylates 'Ser-36' of histone H2B (H2BS36ph), leading to promote transcription. Acts as a key regulator of cell growth and proliferation by phosphorylating TSC2, RPTOR and ATG1: in response to nutrient limitation, negatively regulates the mTORC1 complex by phosphorylating RPTOR component of the mTORC1 complex and by phosphorylating and activating TSC2. In response to nutrient limitation, promotes autophagy by phosphorylating and activating ULK1. AMPK also acts as a regulator of circadian rhythm by mediating phosphorylation of CRY1, leading to destabilize it. May regulate the Wnt signaling pathway by phosphorylating CTNNB1, leading to stabilize it. Also has tau-protein kinase activity: in response to amyloid beta A4 protein (APP) exposure, activated by CAMKK2, leading to phosphorylation of MAPT/TAU; however the relevance of such data remains unclear in vivo. Also phosphorylates CFTR, EEF2K, KLC1, NOS3 and SLC12A1.
Sequence similarities	Belongs to the protein kinase superfamily. CAMK Ser/Thr protein kinase family. SNF1 subfamily. Contains 1 protein kinase domain.
Domain	The AIS (autoinhibitory sequence) region some sequence similarity with the ubiquitin-associated domains and represses kinase activity.
Post-translational modifications	Ubiquitinated. Phosphorylated at Thr-183 by STK11/LKB1 in complex with STE20-related adapter-alpha (STRADA) pseudo kinase and CAB39. Also phosphorylated at Thr-183 by CAMKK2; triggered by a rise in intracellular calcium ions, without detectable changes in the AMP/ATP ratio. CAMKK1 can also phosphorylate Thr-183, but at a much lower level. Dephosphorylated by protein phosphatase 2A and 2C (PP2A and PP2C). Phosphorylated by ULK1 and ULK2; leading to negatively regulate AMPK activity and suggesting the existence of a regulatory feedback loop between ULK1, ULK2 and AMPK.
Cellular localization	Cytoplasm. Nucleus. In response to stress, recruited by p53/TP53 to specific promoters.

Images



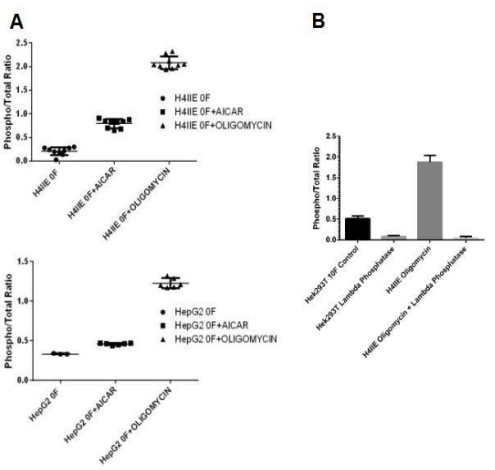
Specificity of Signal by Immunocytochemistry

H4IIE cells were seeded on glass coverslips and allowed to adhere for a few hours. Cells were then serum starved overnight and treated the next day with 1 µM oligomycin (left) or DMSO (right). Levels of AMPKa total and phosphorylated protein at T172/183 were measured following this protocol. The total AMPKa signal is shown in green and AMPKa p172 in red. The left panel shows up-regulation of phosphorylation levels due to oligomycin treatment.



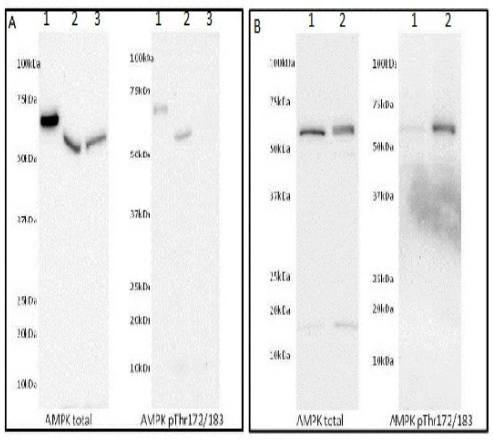
Dynamic range of AMPKa total and pT172

Cells were seeded the day before fixation at the specified cell density and allowed to adhere overnight. Cells were then fixed the next day and signal was obtained using this kit as described. In this experiment, the Hek293T cells were instead permeabilized with methanol at -20°C due to their sensitivity to antigen retrieval. Total AMPKa and AMPKa pT172 are shown after background subtraction.



Specificity of Signal by In Cell ELISA

(A) H4IIE and HepG2 cells were seeded on amine coated plates within the working range of the assay the day before fixation. Levels of total AMPKa and phosphorylated protein at T172/183 were measured after serum starvation and treatment with AICAR (**ab120358**) or oligomycin. Normalized signal intensities were ratio to show the effect of treatment on the phosphorylation status of AMPK.
(B) H4IIE cells treated with oligomycin and untreated Hek293T were fixed on a 96 well plates at densities within the working range of the assay. After fixation, cells were permeabilized with methanol at -20°C for 30 minutes and treated with and without Lamdba Phosphatase at 40°C for 45 minutes on a plate heater. Blocking and antibody incubations were carried out according to this protocol (without the use of Triton X-100). Normalized signal intensities were ratio to show the effect of treatment on the phosphorylation status of AMPK.



Specificity of signal by Western Blot

Western Blot was run on a 4-20% gradient acrylamide gel.
(A) Samples were loaded as follows:
(1) 40ng of AMPKa human recombinant protein,
(2) 40 µg of C2C12 myoblasts serum starved
(3) 40 µg of C2C12 myoblasts in 10% FCS.

(B) Samples were loaded as follows:
(1) 40 µg Hek293T in 10% FCS treated with 1/100 dilution of LP
(2) 40 µg Hek293T in 10% FCS.

AMPKa total membrane was blocked with 5% Milk in TBST, AMPKa pT172 was blocked with 1X Blocking buffer (**ab126587**) in

TBST.

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