abcam

Product datasheet

Human AMPK alpha 1 ELISA Kit ab181422

SimpleStep ELISA®

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Overview

Product name Human AMPK alpha 1 ELISA Kit

Detection method Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%
HEK293T	8			7%

Inter-assay

Sample	n	Mean	SD	CV%
HEK293T	3			11%

Sample type Cell culture extracts, Adherent cells, Suspension cells, Tissue Extracts

Assay type Sandwich (quantitative)

Sensitivity 0.452 ng/ml

Range 1.6 ng/ml - 100 ng/ml

Recovery

Sample specific recovery

Sample type	Average %	Range
Cell culture media	98	90% - 105%

Assay time 1h 30m

Assay duration One step assay

Species reactivity **Reacts with:** Human

Product overview Human AMPK alpha 1 ELISA kit (**ab181423**) is a single-wash 90 min sandwich ELISA designed for the quantitative measurement of AMPK alpha 1 protein in cell and tissue extracts. It uses our proprietary SimpleStep ELISA® technology. Quantitate human AMPK-alpha 1 with 452 pg/mL sensitivity.

SimpleStep ELISA® technology employs capture antibodies conjugated to an affinity tag that is

recognized by the monoclonal antibody used to coat our SimpleStep ELISA® plates. This approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. See the SimpleStep ELISA® protocol summary in the image section for further details. Our SimpleStep ELISA® technology provides several benefits:

-Single-wash protocol reduces assay time to 90 minutes or less
-High sensitivity, specificity and reproducibility from superior antibodies
-Fully validated in biological samples
-96-wells plate breakable into 12 x 8 wells strips

A 384-well SimpleStep ELISA® microplate (**ab203359**) is available to use as an alternative to the 96-well microplate provided with SimpeStep ELISA® kits.

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 (alpha) and two regulatory subunits (beta and gamma). Each subunit has different isoforms (alpha 1, alpha 2, beta 1, beta 2, gamma 1, gamma 2, gamma 3) with differential tissue expression, cellular localization and functionality. Human AMPK has a 98.7% sequence similarity with mouse AMPK and a 99% sequence similarity with rat AMPK. It has been hypothesized that when ADP or AMP are present at high levels, these nucleotides bind directly to the gamma subunit, leading to a conformational change that allows phosphorylation of Thr172 at the alpha subunit. Phosphorylation of AMPK alpha activates the kinase, which leads to downstream effects concerted to increase catabolic pathway and suppress anabolic pathways in order to restore levels of cellular ATP and ultimately control cell fate.

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

Microplate

Properties

Storage instructions

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

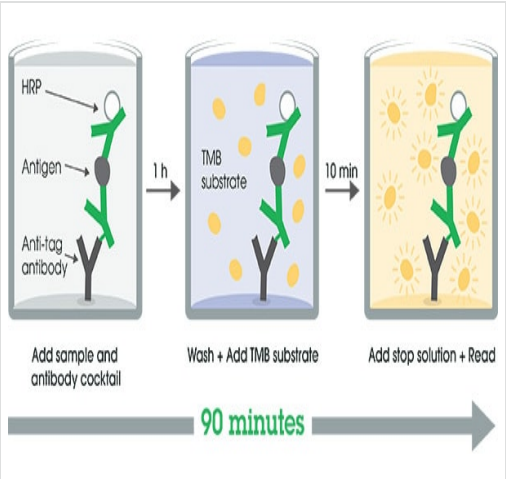
Components	
Plate Seals	1 x unit
10X Wash Buffer PT (**ab206977**)	1 x 20ml
5X Cell Extraction Buffer PTR (**ab193970**)	1 x 10ml
TMB Development Solution	1 x 12ml
Stop Solution	1 x 12ml
SimpleStep Pre-Coated 96-Well Microplate (**ab206978**)	1 x unit
10X Human AMPK-alpha Capture Antibody	1 x 600µl

Components	
10X Human AMPK-alpha Detector Antibody	1 x 600µl
Human AMPK-alpha Lyophilized Recombinant Protein	2 x 1vial
Sample Diluent NS (**ab193972**)	1 x 12ml
Antibody Diluent 5BI	1 x 6ml
50X Cell Extraction Enhancer Solution (**ab193971**)	1 x 1ml

Relevance

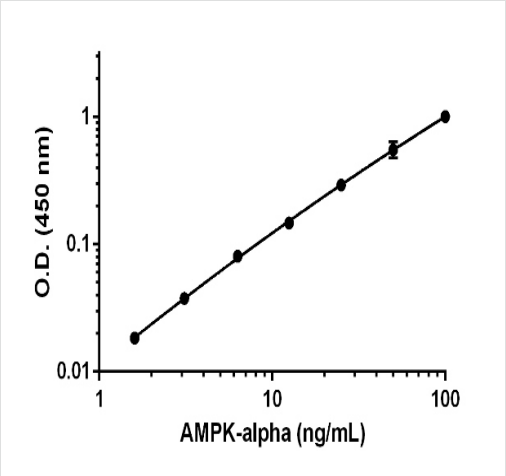
Catalytic subunit of AMP-activated protein kinase (AMPK), an energy sensor protein kinase that plays a key role in regulating cellular energy metabolism. In response to reduction of intracellular ATP levels, AMPK activates energy-producing pathways and inhibits energy-consuming processes: inhibits protein, 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.

Images



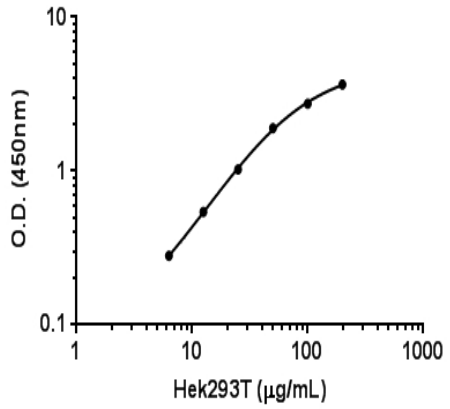
Other - Human AMPK alpha 1 ELISA Kit (ab181422)

SimpleStep ELISA technology allows the formation of the antibody-antigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.



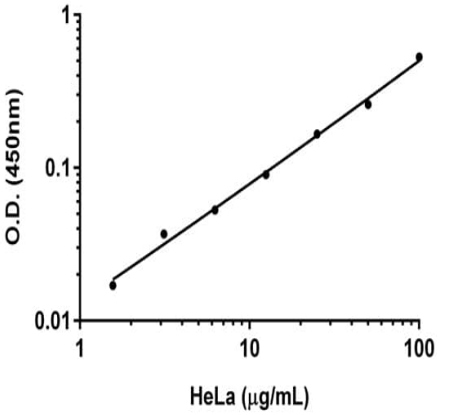
Example of AMPK-alpha 1 standard curve

Background-subtracted data values (mean +/- SD) are graphed.



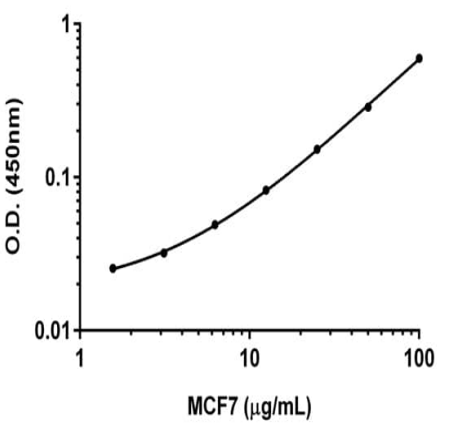
Titration of HEK293T lysate within the working range of the AMPK-alpha 1 assay

Background-subtracted data values (mean +/- SD) are graphed.



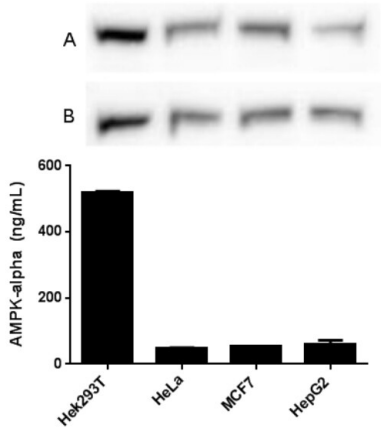
Titration of HeLa lysate within the working range of the AMPK-alpha 1 assay

Background-subtracted data values (mean +/- SD) are graphed.



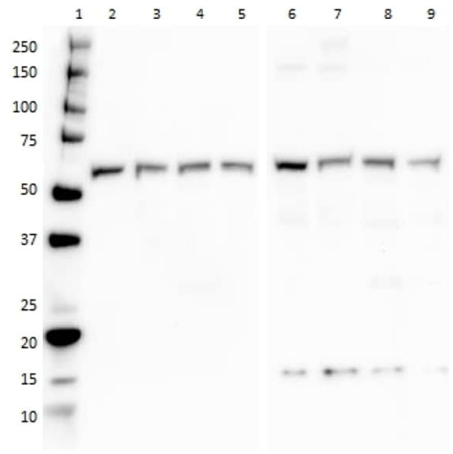
Titration of MCF7 lysate within the working range of the AMPK-alpha 1 assay

Background-subtracted data values (mean +/- SD) are graphed.



Comparative Quantitation of AMPK-alpha 1 expression between Western Blot and SimpleStep ELISA in different cell lines

Comparative Quantitation of AMPK-alpha 1 expression between Western Blot and SimpleStep ELISA in different cell lines. Interpolated values of AMPK-alpha 1 are plotted for the indicated cell lines at 100 µg/mL lysate loading. Corresponding Western Blot bands at 40 ug per lane loading are shown for the capture (A) and detector (B) antibodies. Hek293T cells show significantly higher levels of AMPK-alpha 1 both by western blot and by SimpleStep ELISA



AMPK-alpha 1 expression by Western Blot in different cell lines

Endogenous levels of AMPK-alpha 1 were measured from various cell extracts on western blot using the capture and detector antibodies provided in the kit. Cells were extracted using the protocol outlined in Section 11 and supplemented with SDS sample buffer prior to loading. Samples were loaded as follows: (1) 40 µg Hek293T, (2) 40 µg HeLa, (3) 40 µg MCF7, and (4) 40 µg HepG2. Samples 2-5 were blotted with the detector antibody, and samples 6-9 were blotted with the capture antibody. Blots were performed under reducing conditions. Membranes were blocked with 5% Milk in TBS + 0.1% Tween-20 (TBST) for 1 hour at room temperature. Primary antibodies were incubated in 1% Milk in TBST overnight. Secondary antibodies (goat anti-rabbit HRP for detector and goat anti-mouse HRP for capture) were incubated in 1X Blocking Buffer (**ab126587**) for 2 hours at room temperature.

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