abcam

ab110336

Anti-PDK4 antibody [1C2BG5]

KO Validated

Key facts

Isotype	IgG2a
Host species	Mouse
Storage buffer	pH: 7.5 Preservative: 0.02% Sodium azide Constituents: HEPES buffered saline
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	1C2BG5
Purification technique	Proprietary technique
Specificity	This antibody does not cross-react with other isoforms of Human PDK (i.e. PDK1, 2 or 3).
Light chain type	kappa
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
Purification notes	Produced in vitro using hybridomas grown in serum-free medium, and then purified by biochemical fractionation. Purity >95% by SDS-PAGE.

Reactivity data

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1 µg for 10^6 Cells
Notes	ab170191 - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.

WB

Tested

Species	Human
Dilution info	1 µg/mL
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	10 µg/mL
Notes	-

Target data

See full target information PDK4

Function

Kinase that plays a key role in regulation of glucose and fatty acid metabolism and homeostasis via phosphorylation of the pyruvate dehydrogenase subunits PDHA1 and PDHA2. This inhibits pyruvate dehydrogenase activity, and thereby regulates metabolite flux through the tricarboxylic acid cycle, down-regulates aerobic respiration and inhibits the formation of acetyl-coenzyme A from pyruvate. Inhibition of pyruvate dehydrogenase decreases glucose utilization and increases fat metabolism in response to prolonged fasting and starvation. Plays an important role in maintaining normal blood glucose levels under starvation, and is involved in the insulin signaling cascade. Via its regulation of pyruvate dehydrogenase activity, plays an important role in maintaining normal blood pH and in preventing the accumulation of ketone bodies under starvation. In the fed state, mediates cellular responses to glucose levels and to a high-fat diet. Regulates both fatty acid oxidation and de novo fatty acid biosynthesis. Plays a role in the generation of reactive oxygen species. Protects detached epithelial cells against anoikis. Plays a role in cell proliferation via its role in regulating carbohydrate and fatty acid metabolism.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

Want a custom formulation?
This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	PDK4 also known as Pyruvate Dehydrogenase Kinase Isozyme 4 plays an essential role in regulating mitochondrial energy metabolism. PDK4 phosphorylates the pyruvate dehydrogenase complex controlling the transformation of pyruvate to acetyl-CoA a critical step in energy production. The protein has a molecular weight of around 49 kDa. PDK4 expression occurs in various tissues with high levels observed in skeletal muscle heart and liver reflecting its role in energy metabolic processes.
Biological function summary	The enzyme functions to inhibit the pyruvate dehydrogenase complex by adding phosphate groups which reduces the conversion of pyruvate to acetyl-CoA and shifts energy production from carbohydrates to fats especially during fasting or prolonged exercise. PDK4 operates as a homodimer and is often part of a larger regulatory system balancing glucose and fatty acid oxidation in response to nutrient availability.
Pathways	PDK4 is integral to the regulation of both glucose metabolism and fatty acid oxidation. The enzyme interacts closely with the insulin signaling pathway where it modulates glucose uptake and utilization based on hormonal signals. Additionally PDK4 is a part of the hypoxia-inducible factor-1 (HIF-1) pathway where it plays a role in cellular response to low oxygen levels interacting with related proteins such as pyruvate dehydrogenase (PDH).
Associated diseases and disorders	PDK4's activity is linked to metabolic conditions like diabetes and obesity where its altered expression can lead to insulin resistance by affecting glucose utilization. Furthermore PDK4 is associated with cardiovascular diseases through its influence on cardiac energy metabolism and its interaction with other proteins such as PDH contributing to heart dysfunction when deregulated.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

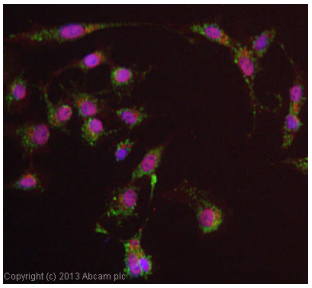
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

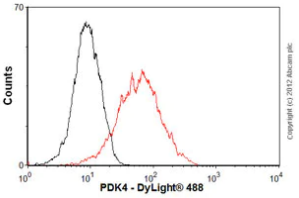
7 product images



Immunocytochemistry/ Immunofluorescence - Anti-PDK4 antibody [1C2BG5] (ab110336)

PDK4 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-PDK4 antibody

ICC/IF image of ab110336 stained HepG2 cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab110336, 10µg/ml) overnight at +4°C. The secondary antibody (green) was ab96879, DyLight® 488 goat anti-mouse IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

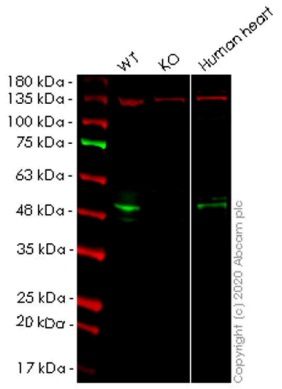


Flow Cytometry (Intracellular) - Anti-PDK4 antibody [1C2BG5] (ab110336)

PDK4 Flow Cytometry (Intracellular) staining using mouse Anti-PDK4 antibody

Overlay histogram showing HepG2 cells stained with ab110336 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells

were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab110336, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2a [ICIGG2A] (ab91361, 1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.



Western blot - Anti-PDK4 antibody [1C2BG5] (ab110336)

PDK4 Western blot staining using mouse Anti-PDK4 antibody

Lanes 1-3: Merged signal (red and green). Green - ab110336 observed at 48 kDa. Red - loading control, ab129002 observed at 124 kDa.
ab110336 Anti-PDK4 antibody [1C2BG5] was shown to specifically react with PDK4 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab261805 (knockout cell lysate ab257217) was used. Wild-type and PDK4 knockout samples were subjected to SDS-PAGE. ab110336 and Anti-Vinculin antibody [EPR8185] - Loading Control (ab129002) were incubated overnight at 4°C at 1 in 500 dilution and 1 in 1000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (ab216772) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-PDK4 antibody [1C2BG5] (ab110336) at 1/500 dilution

Lane 1:
Wild-type HeLa cell lysate at 20 µg

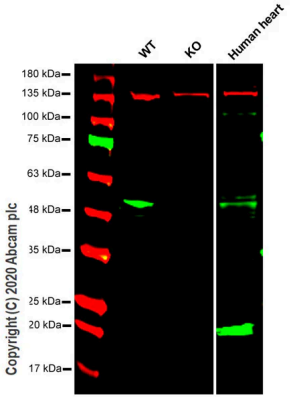
Lane 2:
PDK4 knockout HeLa cell lysate at 20 µg

Lane 3:
Human heart tissue lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 46 kDa

Observed band size: 48 kDa



Western blot - Anti-PDK4 antibody [1C2BG5] (ab110336)

PDK4 Western blot staining using mouse Anti-PDK4 antibody

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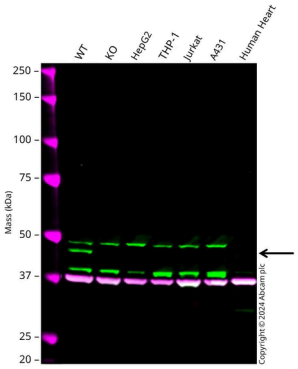
Lane 2:
PDK4 knockout HeLa cell lysate at 20 µg

Lane 3:
Human heart tissue lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 46 kDa

Observed band size: 48 kDa



Western blot - Anti-PDK4 antibody [1C2BG5] (ab110336)

PDK4 Western blot staining using mouse Anti-PDK4 antibody

Western blot: Anti-PDK4 antibody [1C2BG5] (ab110336) staining at 1 ug/ml, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (ab181602) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab110336 was shown to bind specifically to PDK4. A band was observed at 45 kDa in wild-type HeLa cell lysates with no signal observed at this size in PDK4 knockout cell line. To generate this image, wild-type and PDK4 knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse IgG H&L 800CW and Goat anti-Rabbit IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-PDK4 antibody [1C2BG5] (ab110336) at 1 µg/mL

Lane 1:
Wild-type HeLa cell lysate at 20 µg

Lane 2:
PDK4 knockout HeLa cell lysate at 20 µg

Lane 3:
HepG2 cell lysate at 20 µg

Lane 4:
THP-1 cell lysate at 20 µg

Lane 5:
Jurkat cell lysate at 20 µg

Lane 6:
A431 cell lysate at 20 µg

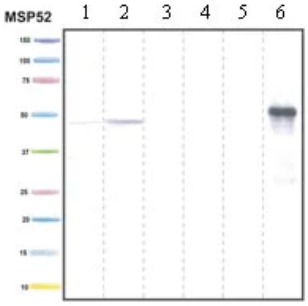
Lane 7:
Human Heart cell lysate at 20 µg

Secondary

All lanes:
Goat anti-Mouse IgG H&L 800CW and Goat anti-Rabbit IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Observed band size: 45 kDa



Western blot - Anti-PDK4 antibody [1C2BG5] (ab110336)

All lanes:
Western blot - Anti-PDK4 antibody [1C2BG5] (ab110336) at 1 µg/mL

Lane 1:
HepG2 whole cell lysate at 10 µg

Lane 2:
Human heart tissue at 10 µg

Lane 3:
Recombinant Human PDK1 at 0.01 µg

Lane 4:
Recombinant Human PDK2 at 0.01 µg

Lane 5:
Recombinant Human PDK3 at 0.01 µg

Lane 6:
Recombinant Human PDK4 at 0.01 µg

Predicted band size: 46 kDa

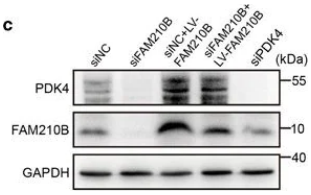


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Western blot - Anti-PDK4 antibody [1C2BG5] (ab110336)

PDK4 western blot using anti-PDK4 antibody [1C2BG5] ab110336. Publication image and figure legend from Sun, S., Liu, J., et al., 2017, Cell Death Dis, PubMed 28594398.

ab110336 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab110336 please see the product overview.

FAM210B knockdown cells show decreased PDK4 expression. (a) Heat map of the top 25 differentially regulated genes in siFAM210B and negative control A549 cells. (b) Quantitative PCR analyses of the relative expression of the indicated genes in siFAM210B normalized to negative control SKOV3 cells. (c) Immunoblot for PDK4 in the indicated treated SKOV3 cells. (d) Schematically depicted PDK function in SKOV3 cell metabolism. (e) Western blot analysis of the phosphorylation levels of PDH-E1α in the indicated treated SKOV3 cells. (f) Normalized OCR and (g) normalized ECAR in siPDK4 (n=6) and negative control SKOV3 cells (n=8 wells)

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