abcam

Product datasheet

Anti-PI 3 Kinase p85 alpha antibody [EPR18702] - BSA and Azide free ab223792

KO VALIDATED Recombinant RabMAb

8 Images

Overview

Product name	Anti-PI 3 Kinase p85 alpha antibody [EPR18702] - BSA and Azide free
Description	Rabbit monoclonal [EPR18702] to PI 3 Kinase p85 alpha - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt, ICC/IF, IP, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Rat PI 3 Kinase p85 alpha aa 600-700. The exact sequence is proprietary. Database link: Q63787
Positive control	WB: Human PI3K p85 alpha full length recombinant protein; Human fetal liver, fetal heart and fetal kidney lysates; HeLa, HepG2, MCF7, Raji, Jurkat, C6, RAW 264.7, PC-12 and NIH/3T3 whole cell lysates; Mouse brain, heart, kidney and spleen lysates; Rat brain, heart, kidney and spleen lysates. ICC/IF: HepG2 and NIH/3T3 cells. Flow Cyt: NIH/3T3 cells; IP: MCF7 whole cell lysate.
General notes	Ab223792 is the carrier-free version of ab191606. This format is designed for use in antibody labeling, including fluorochromes, metal isotopes, oligonucleotides, enzymes. Our carrier-free formats are supplied in a buffer free of BSA, sodium azide and glycerol for higher conjugation efficiency. Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. ab223792 is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm. *Maxpar® is a trademark of Fluidigm Canada Inc.* This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information see here. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit

monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough.

Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility.

We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee.

In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for.

We are also updating the applications & species that this product has been "predicted to work with," however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, as well as customer reviews and Q&As.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR18702
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab223792** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

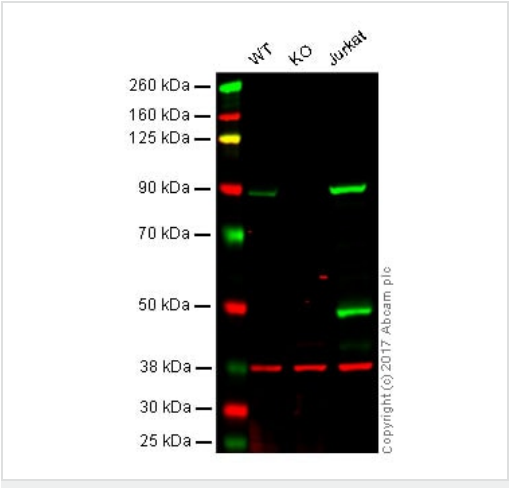
Application	Abreviews	Notes
Flow Cyt		Use at an assay dependent concentration. ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.

Application	Abreviews	Notes
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 85,46 kDa (predicted molecular weight: 84 kDa).

Target

Function	Binds to activated (phosphorylated) protein-Tyr kinases, through its SH2 domain, and acts as an adapter, mediating the association of the p110 catalytic unit to the plasma membrane. Necessary for the insulin-stimulated increase in glucose uptake and glycogen synthesis in insulin-sensitive tissues.
Tissue specificity	Isoform 2 is expressed in skeletal muscle and brain, and at lower levels in kidney and cardiac muscle. Isoform 2 and isoform 4 are present in skeletal muscle (at protein level).
Sequence similarities	Belongs to the PI3K p85 subunit family. Contains 1 Rho-GAP domain. Contains 2 SH2 domains. Contains 1 SH3 domain.
Domain	The SH3 domain mediates the binding to CBLB, and to HIV-1 Nef.
Post-translational modifications	Polyubiquitinated in T-cells by CBLB; which does not promote proteasomal degradation but impairs association with CD28 and CD3Z upon T-cell activation. Phosphorylated. Dephosphorylated by PTPRJ.

Images



Western blot - Anti-PI 3 Kinase p85 alpha antibody [EPR18702] - BSA and Azide free (ab223792)

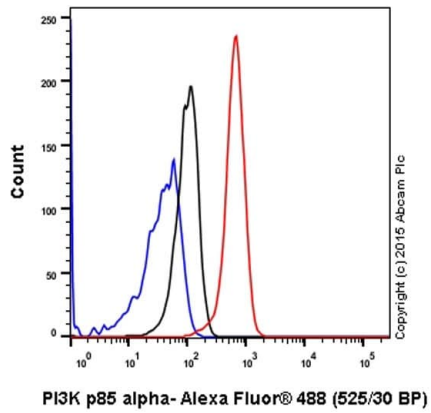
This WB data was generated using the same anti-PI 3 Kinase p85 alpha antibody clone [EPR18702] in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (cat# ab191606).

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: PIK3R1 knockout HAP1 whole cell lysate (20 µg)
Lane 3: Jurkat whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab191606 observed at 90 kDa. Red - loading control, ab9484, observed at 37 kDa.

ab191606 was shown to specifically react with PIK3R1 when PIK3R1 knockout samples were used. Wild-type and PIK3R1 knockout samples were subjected to SDS-PAGE. Ab191606 and ab9484 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary

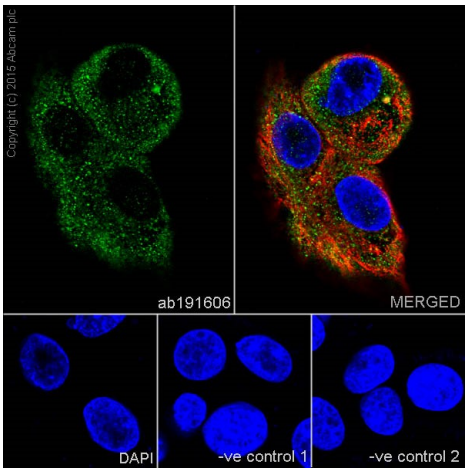
antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Flow Cytometry - Anti-PI 3 Kinase p85 alpha antibody [EPR18702] - BSA and Azide free (ab223792)

Overlay histogram showing HepG2 cells fixed in 4% PFA and stained with ab191606 at a dilution of 1/80 (red line). The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit at a dilution of 1/500. Rabbit monoclonal IgG (ab172730) was used as an isotype control (black line) and cells incubated in the absence of both primary and secondary antibody were used as a negative control (blue line).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab191606).



Immunocytochemistry/ Immunofluorescence - Anti-PI 3 Kinase p85 alpha antibody [EPR18702] - BSA and Azide free (ab223792)

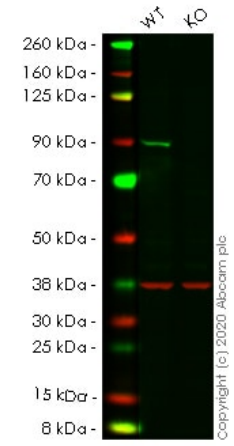
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HepG2 (Human liver hepatocellular carcinoma cell line) cells labeling PI3K p85 with ab191606 at 1/250 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on HepG2 cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody - Loading Control (ab7291) at 1/1000 dilution and Goat Anti-Mouse IgG (AlexaFluor®594) preadsorbed (ab150120) at 1/1000 dilution (red).

The negative controls are as follows:

-ve control 1: ab191606 at 1/500 dilution followed by ab150120 at 1/1000 dilution.

-ve control 2: ab7291 at 1/1000 dilution followed by ab150077 at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab191606).



Western blot - Anti-PI 3 Kinase p85 alpha antibody [EPR18702] - BSA and Azide free (ab223792)

All lanes : Anti-PI 3 Kinase p85 alpha antibody [EPR18702] (ab191606) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : PIK3R1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

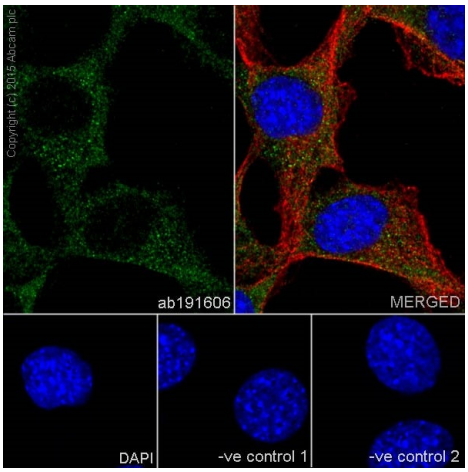
Performed under reducing conditions.

Predicted band size: 84 kDa
Observed band size: 90 kDa
why is the actual band size different from the predicted?

This data was developed using the same antibody clone in a different buffer formulation (ab191606).

Lanes 1- 2: Merged signal (red and green). Green - ab191606 observed at 90 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

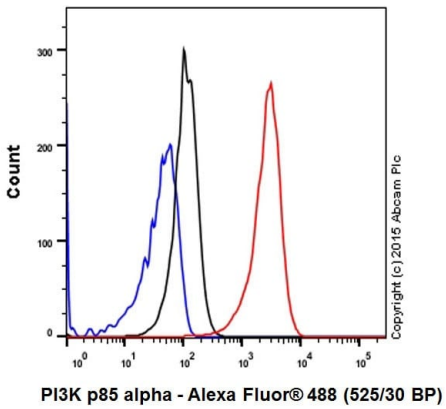
ab191606 was shown to react with PI 3 Kinase p85 alpha in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab265116 (knockout cell lysate ab257029) was used. Wild-type HeLa and PIK3R1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab191606 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-PI 3 Kinase p85 alpha antibody [EPR18702] - BSA and Azide free (ab223792)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 100% Methanol permeabilized NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling PI3K p85 with ab191606 at 1/250 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on NIH/3T3 cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody - Loading Control (ab7291) at 1/1000 dilution and Goat Anti-Mouse IgG (AlexaFluor®594) preadsorbed (ab150120) at 1/1000 dilution (red).
The negative controls are as follows:
-ve control 1: ab191606 at 1/500 dilution followed by ab150120 at 1/1000 dilution.
-ve control 2: ab7291 at 1/1000 dilution followed by ab150077 at 1/1000 dilution.

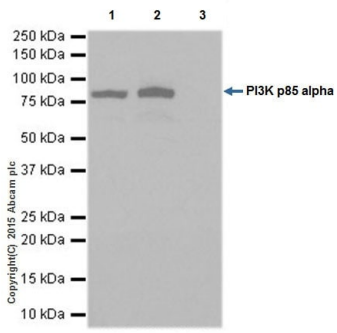
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab191606).



Flow Cytometry - Anti-PI 3 Kinase p85 alpha antibody [EPR18702] - BSA and Azide free (ab223792)

Flow cytometric analysis of 4% paraformaldehyde-fixed NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling PI3K p85 with ab191606 at 1/150 dilution (red) compared with a Rabbit IgG,monoclonal - Isotype control (ab172730) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti Rabbit IgG (Alexa Fluor® 488) at 1/500 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab191606).



Immunoprecipitation - Anti-PI 3 Kinase p85 alpha antibody [EPR18702] - BSA and Azide free (ab223792)

PI3K p85 was immunoprecipitated from 1mg of MCF7 (Human breast adenocarcinoma cell line) whole cell lysate with ab191606 at 1/50 dilution. Western blot was performed from the immunoprecipitate using ab191606 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

Lane 1: MCF7 whole cell lysate, 10µg (Input).

Lane 2: ab191606 IP in MCF7 whole cell lysate.

Lane 3: Rabbit IgG, monoclonal - Isotype Control (ab172730) instead of ab191606 in MCF7 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 5 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab191606).

Anti-PI 3 Kinase p85 alpha antibody [EPR18702] - BSA and Azide free (ab223792)

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