abcam

Product datasheet

Anti-PI 3 Kinase p85 alpha antibody [EPR18702] ab191606

KO VALIDATED Recombinant RabMAb

★★★★★ 2 Abreviews 176 References 12 Images

Overview

Product name	Anti-PI 3 Kinase p85 alpha antibody [EPR18702]
Description	Rabbit monoclonal [EPR18702] to PI 3 Kinase p85 alpha
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, ICC/IF, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human, African green monkey
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
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 (intra): NIH/3T3 cells; IP: MCF7 whole cell lysate.
General notes	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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR18702
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab191606 in the following tested applications.

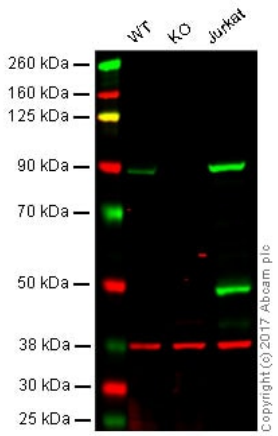
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/150. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ (2)	1/1000. Detects a band of approximately 85,46 kDa (predicted molecular weight: 84 kDa).
ICC/IF		1/250.
IP		1/50.

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

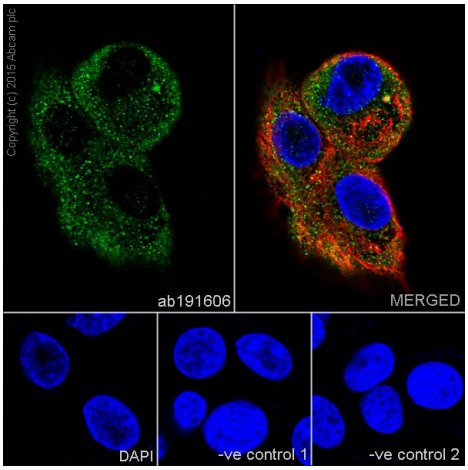


Western blot - Anti-PI 3 Kinase p85 alpha antibody [EPR18702] (ab191606)

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.



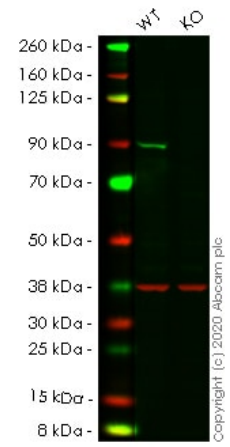
Immunocytochemistry/ Immunofluorescence - Anti-PI 3 Kinase p85 alpha antibody [EPR18702] (ab191606)

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.



Western blot - Anti-PI 3 Kinase p85 alpha antibody [EPR18702] (ab191606)

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

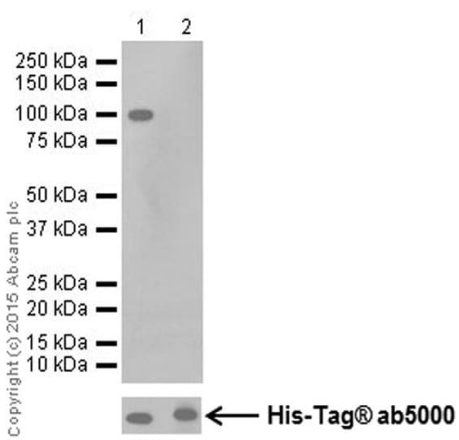
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 84 kDa
Observed band size: 90 kDa

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.



Western blot - Anti-PI 3 Kinase p85 alpha antibody [EPR18702] (ab191606)

All lanes : Anti-PI 3 Kinase p85 alpha antibody [EPR18702] (ab191606) at 1/20000 dilution

Lane 1 : Human PI3K p85 alpha full length recombinant protein
Lane 2 : Human PI3K p85 beta full length recombinant protein

Lysates/proteins at 0.01 µg per lane.

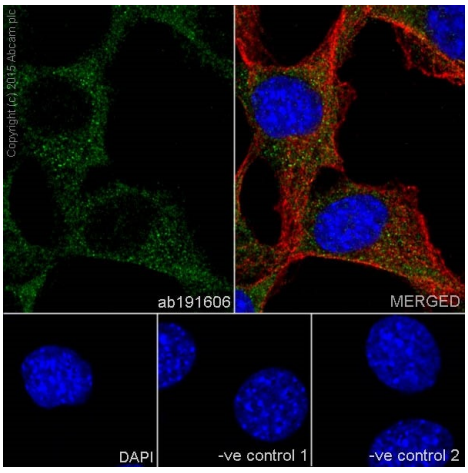
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 84 kDa

Exposure time: 5 seconds

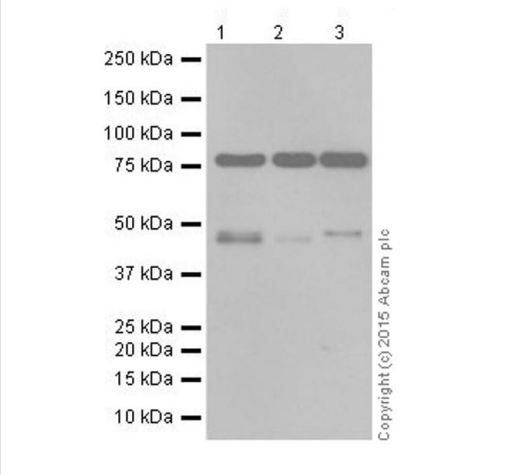
Blocking/Dilution buffer: 5% NFDM/TBST.

Human PI3K p85 alpha full length recombinant protein contain aa1-724 with a His-Tag® (Cat#**ab84769**). Human PI3K p85 beta full length recombinant protein contain aa1-728 with a His-Tag® (Cat#**ab125568**).



Immunocytochemistry/ Immunofluorescence - Anti-PI 3 Kinase p85 alpha antibody [EPR18702] (ab191606)

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.



Western blot - Anti-PI 3 Kinase p85 alpha antibody [EPR18702] (ab191606)

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

Lane 1 : Human fetal liver lysate
Lane 2 : Human fetal heart lysate
Lane 3 : Human fetal kidney lysate

Lysates/proteins at 10 µg per lane.

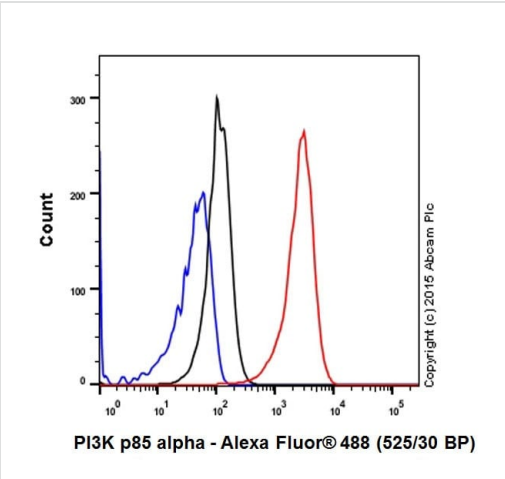
Secondary
All lanes : Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 84 kDa
Observed band size: 46,85 kDa

Exposure time: 3 minutes

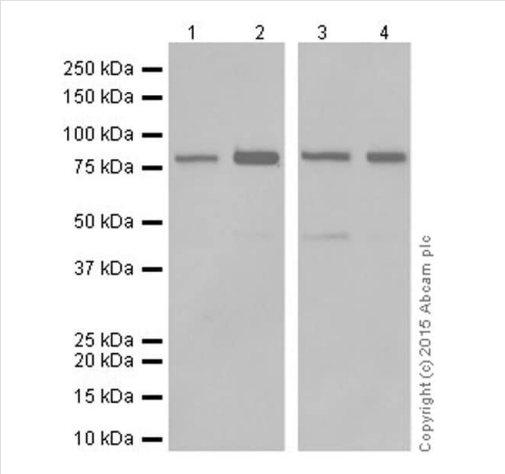
Blocking/Dilution buffer: 5% NFDM/TBST.

The molecular weight observed is consistent with what have been described in the literatures (PMID: 8921377, 12649157).



Flow Cytometry (Intracellular) - Anti-PI 3 Kinase p85 alpha antibody [EPR18702] (ab191606)

Intracellular 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.



Western blot - Anti-PI 3 Kinase p85 alpha antibody [EPR18702] (ab191606)

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

Lane 1 : HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate
Lane 2 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate
Lane 3 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate
Lane 4 : Raji (Human Burkitt's lymphoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

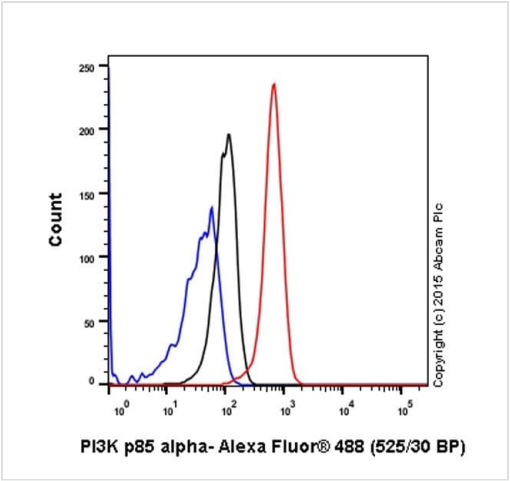
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 84 kDa
Observed band size: 46,85 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

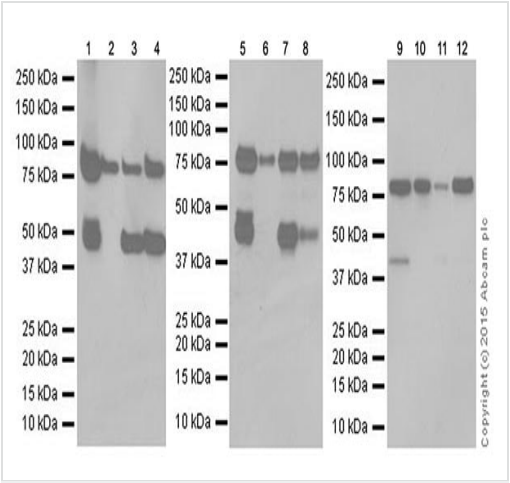
Exposure time: Lane 1 and 2: 10 seconds; Lane 3 and 4: 3 seconds.

The molecular weight observed is consistent with what have been described in the literatures (PMID: 8921377, 12649157).



Flow Cytometry (Intracellular) - Anti-PI 3 Kinase p85 alpha antibody [EPR18702] (ab191606)

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).



Western blot - Anti-PI 3 Kinase p85 alpha antibody [EPR18702] (ab191606)

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

Lane 1 : Mouse brain lysate
Lane 2 : Mouse heart lysate
Lane 3 : Mouse kidney lysate
Lane 4 : Mouse spleen lysate
Lane 5 : Rat brain lysate
Lane 6 : Rat heart lysate
Lane 7 : Rat kidney lysate
Lane 8 : Rat spleen lysate
Lane 9 : C6 (Rat glial tumor cell line) whole cell lysate
Lane 10 : RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate
Lane 11 : PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate
Lane 12 : NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

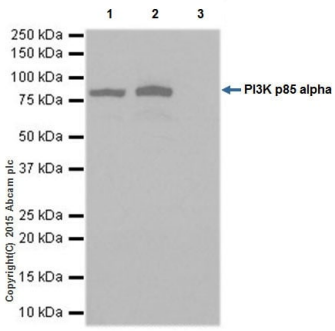
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 84 kDa
Observed band size: 46,85 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1-8: 3 minutes; Lane 9-12: 10 seconds.

The molecular weight observed is consistent with what have been described in the literatures (PMID: 8921377, 12649157).



Immunoprecipitation - Anti-PI 3 Kinase p85 alpha antibody [EPR18702] (ab191606)

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.

Anti-PI 3 Kinase p85 alpha antibody [EPR18702] (ab191606)

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