

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

KO 評価済 リコンビナント RabMAb

★★★★★ **2 Abreviews** **176 References** 画像数 **12**

#### 製品の概要

製品名	Anti-PI 3 Kinase p85 alpha antibody [EPR18702]
製品の詳細	Rabbit monoclonal [EPR18702] to PI 3 Kinase p85 alpha
由来種	Rabbit
アプリケーション	<b>適用あり:</b> Flow Cyt (Intra), WB, ICC/IF, IP
種交差性	<b>交差種:</b> Mouse, Rat, Human, African green monkey
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	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.
特記事項	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p>

#### 製品の特性

製品の状態	Liquid
保存方法	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.
バッファー	<p>pH: 7.2</p> <p>Preservative: 0.01% Sodium azide</p> <p>Constituents: PBS, 40% Glycerol, 0.05% BSA</p>
精製度	Protein A purified
ポリ/モノ	モノクローナル

クローン名 EPR18702  
アイソタイプ IgG

## アプリケーション

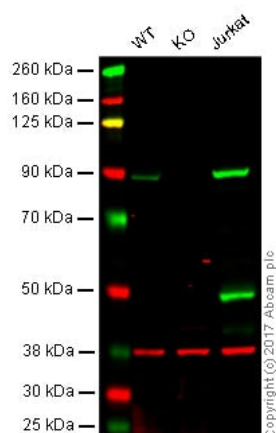
**The Abpromise guarantee** Abpromise保証は、次のテスト済みアプリケーションにおけるab191606の使用に適用されます  
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/150. <b>ab172730</b> - 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.

## ターゲット情報

機能	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.
組織特異性	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).
配列類似性	Belongs to the PI3K p85 subunit family. Contains 1 Rho-GAP domain. Contains 2 SH2 domains. Contains 1 SH3 domain.
ドメイン	The SH3 domain mediates the binding to CBLB, and to HIV-1 Nef.
翻訳後修飾	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.

## 画像



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

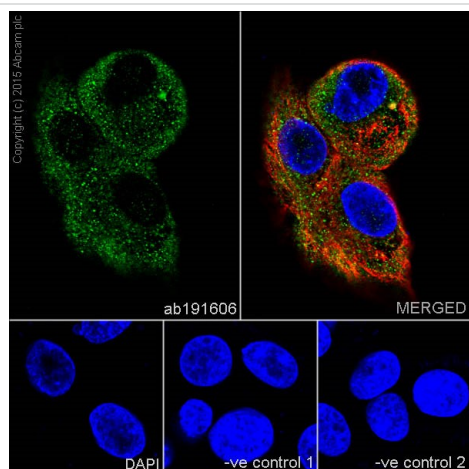
**Lane 1:** Wild type HAP1 whole cell lysate (20 µg)

**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.



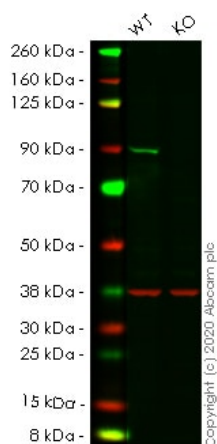
Immunocytochemistry/ Immunofluorescence - Anti-PI 3 Kinase p85 alpha antibody [EPR18702] (ab191606)

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.

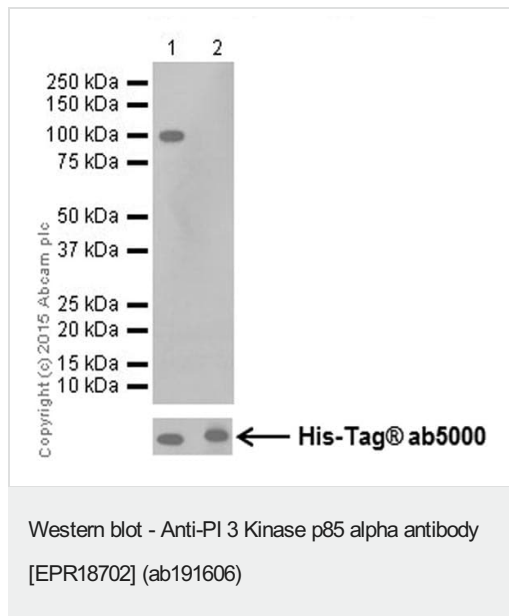
Performed under reducing conditions.

**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.



**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

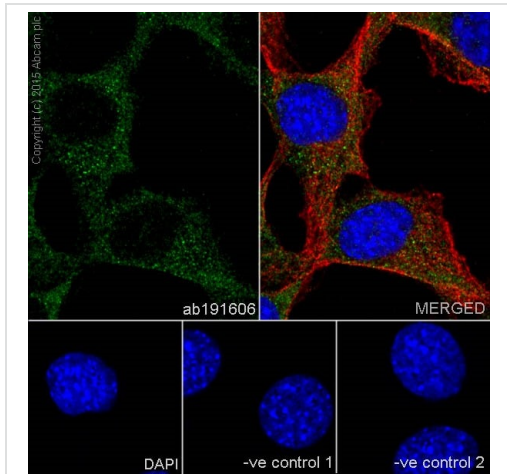
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution

**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](#)).



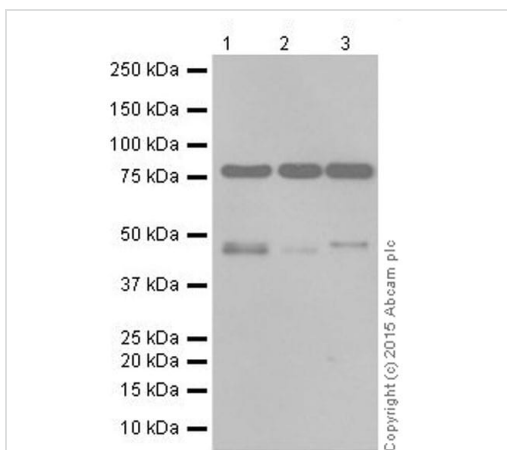
Immunocytochemistry/ Immunofluorescence - Anti-PI 3 Kinase p85 alpha antibody [EPR18702] (ab191606)

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

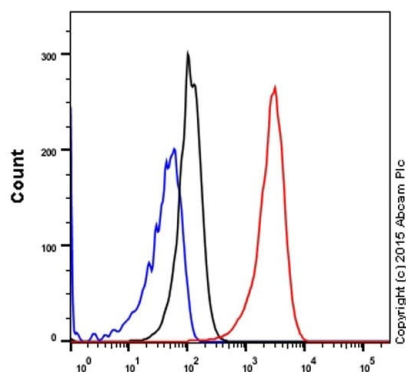
**Predicted band size:** 84 kDa

**Observed band size:** 46,85 kDa

**Exposure time:** 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

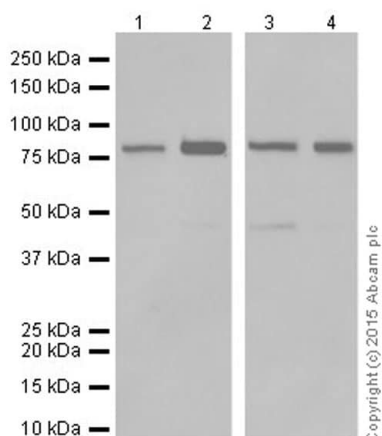
The molecular weight observed is consistent with what have been described in the literatures (PMID: 8921377, 12649157).



PI3K p85 alpha - Alexa Fluor® 488 (525/30 BP)

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

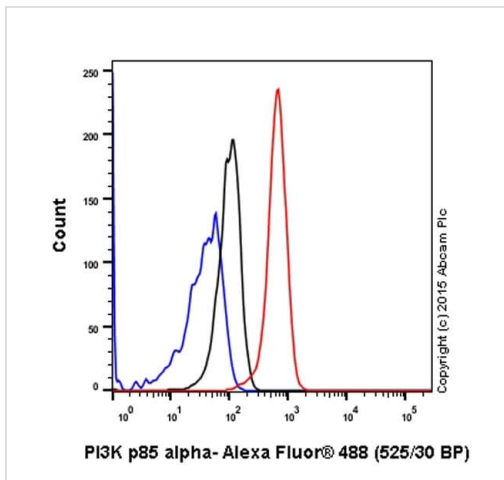
**Predicted band size:** 84 kDa

**Observed band size:** 46,85 kDa

Blocking/Dilution buffer: 5% NFDm/TBST.

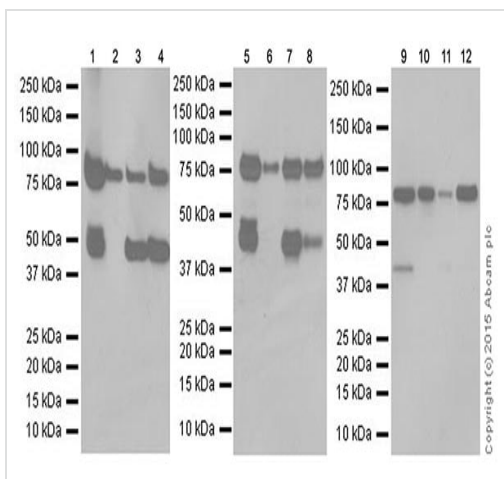
Exposure time: Lane 1 and 2: 10 seconds; Lane 3 and 4: 3 seconds.

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<sup>®</sup> 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

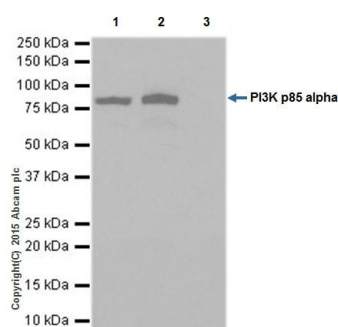


**Observed band size:** 46,85 kDa

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.

Why choose a recombinant antibody?



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Consistent and reproducible results



**Long-term and scalable supply**  
Recombinant technology



**Success from the first experiment**  
Confirmed specificity



**Ethical standards compliant**  
Animal-free production

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

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