abcam

Product datasheet

Anti-PI 3 Kinase p85 beta antibody [EPR18416] ab180967

敲除验证 重组 RabMAb

25 References 12 图像

概述

产品名称	Anti-PI 3 Kinase p85 beta抗体[EPR18416]
描述	兔单克隆抗体[EPR18416] to PI 3 Kinase p85 beta
宿主	Rabbit
经测试应用	**适用于:** IHC-P, WB, ICC/IF, IP **不适用于:** Flow Cyt
种属反应性	**与反应:** Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: HAP1, HeLa, Jurkat, HEK293 cell lysates; Human fetal brain and fetal kidney lysates; Rat brain and spleen lysates; PC12 cell lysate. IHC-P: Human colonic adenocarcinoma, Human colon, rat testis tissues. ICC/IF: HeLa and Jurkat cells. IP: Jurkat cell lysate.
常规说明	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**.

性能

形式	Liquid
存放说明	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.
存储溶液	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR18416

同种型 IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab180967于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/2000. Detects a band of approximately 82 kDa (predicted molecular weight: 82 kDa).
ICC/IF		1/500.
IP		1/80.

应用说明 Is unsuitable for Flow Cyt.

靶标

功能 Regulatory subunit of phosphoinositide-3-kinase (PI3K), a kinase that phosphorylates PtdIns(4,5)P2 (Phosphatidylinositol 4,5-bisphosphate) to generate phosphatidylinositol 3,4,5-trisphosphate (PIP3). PIP3 plays a key role by recruiting PH domain-containing proteins to the membrane, including AKT1 and PDPK1, activating signaling cascades involved in cell growth, survival, proliferation, motility and morphology. Binds to activated (phosphorylated) protein-tyrosine kinases, through its SH2 domain, and acts as an adapter, mediating the association of the p110 catalytic unit to the plasma membrane. Indirectly regulates autophagy (PubMed:23604317). Promotes nuclear translocation of XBP1 isoform 2 in a ER stress- and/or insulin-dependent manner during metabolic overloading in the liver and hence plays a role in glucose tolerance improvement.

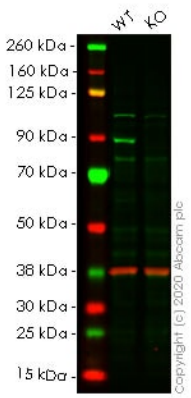
疾病相关 Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome 1

序列相似性 Belongs to the PI3K p85 subunit family.
Contains 1 Rho-GAP domain.
Contains 2 SH2 domains.
Contains 1 SH3 domain.

结构域 The SH2 2 domain is required for interaction with FBXL2 and PTPN13.

翻译后修饰 Phosphorylated in response to signaling from activated receptor-type protein kinases (PubMed:19690332, PubMed:20068231). Dephosphorylated by PTPRJ (PubMed:18348712). Dephosphorylated at Tyr-655 by PTPN13. Phosphorylation of Tyr-655 impairs while its dephosphorylation promotes interaction with FBXL2 and SCF(FBXL2)-mediated polyubiquitination (PubMed:23604317).
Ubiquitinated. Polyubiquitination by the SCF(FBXL2) complex probably promotes proteasomal degradation of PIK3R2.

图片



Western blot - Anti-PI 3 Kinase p85 beta antibody [EPR18416] (ab180967)

All lanes : Anti-PI 3 Kinase p85 beta antibody [EPR18416] (ab180967) at 1/2000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : PIK3R2 knockout HEK-293T cell lysate

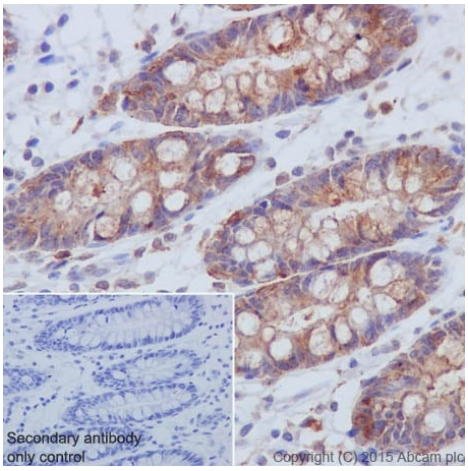
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 82 kDa
Observed band size: 85 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab180967 observed at 85 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab180967 was shown to react with PI 3 Kinase p85 beta in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab266799** (knockout cell lysate **ab257586**) was used. Wild-type HEK-293T and PIK3R2 knockout HEK-293T cell lysates were subjected to SDS-PAGE. ab180967 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 2000 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.

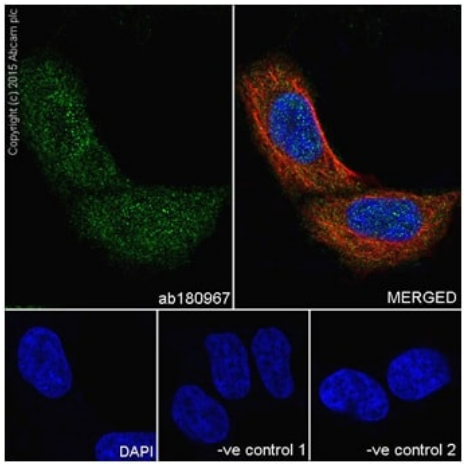


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PI 3 Kinase p85 beta antibody [EPR18416] (ab180967)

Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling PI 3 Kinase p85 beta using ab180967 at 1/100 dilution. A Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) was used as secondary at 1/500 dilution. Cytoplasm staining on epithelial cells of Human colon was observed. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



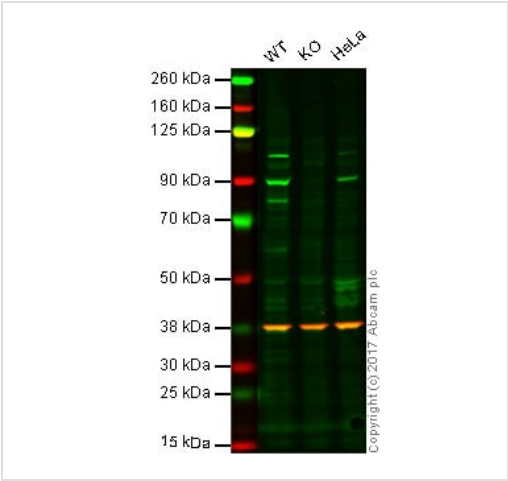
Immunocytochemistry/ Immunofluorescence - Anti-PI 3 Kinase p85 beta antibody [EPR18416] (ab180967)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling PI 3 Kinase p85 beta with ab180967 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing nuclear and cytoplasmic staining on HeLa cell line.

The nuclear counter stain is DAPI (blue).

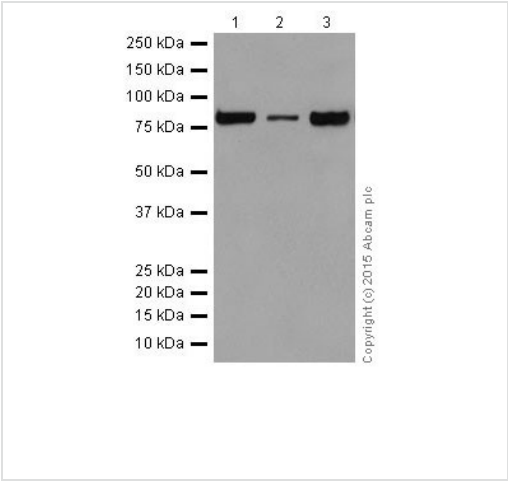
Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).
The negative controls are as follows:
1. ab180967 at 1/500 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution.
2. **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.



Western blot - Anti-PI 3 Kinase p85 beta antibody [EPR18416] (ab180967)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: PIK3R2 knockout HAP1 whole cell lysate (20 µg)
Lane 3: HeLa whole cell lysate (20 µg)
Lanes 1 - 3: Merged signal (red and green). Green - ab180967 observed at 85 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab180967 was shown to specifically react with PIK3R2 (KO) when PIK3R2 (KO) knockout samples were used. Wild-type and PIK3R2 (KO) knockout samples were subjected to SDS-PAGE. Ab180967 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 2000 dilution and 1/10000 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 1/10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-PI 3 Kinase p85 beta antibody [EPR18416] (ab180967)

All lanes : Anti-PI 3 Kinase p85 beta antibody [EPR18416] (ab180967) at 1/2000 dilution

Lane 1 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate
Lane 2 : Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysate
Lane 3 : HEK293 (Human epithelial cells from embryonic kidney) whole cell lysate

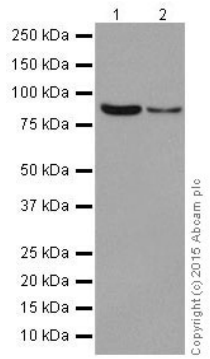
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/1000 dilution

Predicted band size: 82 kDa
Observed band size: 82 kDa

Exposure time: 30 seconds

5% NFDM/TBST: Blocking and diluting buffer.



Western blot - Anti-PI 3 Kinase p85 beta antibody [EPR18416] (ab180967)

All lanes : Anti-PI 3 Kinase p85 beta antibody [EPR18416] (ab180967) at 1/2000 dilution

Lane 1 : Human fetal brain lysate
Lane 2 : Human fetal kidney lysate

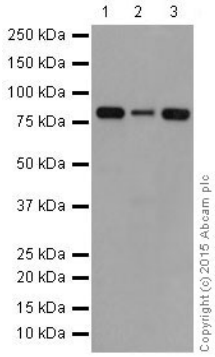
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 82 kDa
Observed band size: 82 kDa

Exposure time: 3 minutes

5% NFDM/TBST: Blocking and diluting buffer.



Western blot - Anti-PI 3 Kinase p85 beta antibody [EPR18416] (ab180967)

All lanes : Anti-PI 3 Kinase p85 beta antibody [EPR18416] (ab180967) at 1/2000 dilution

Lane 1 : Rat brain lysate
Lane 2 : Rat spleen lysate
Lane 3 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate

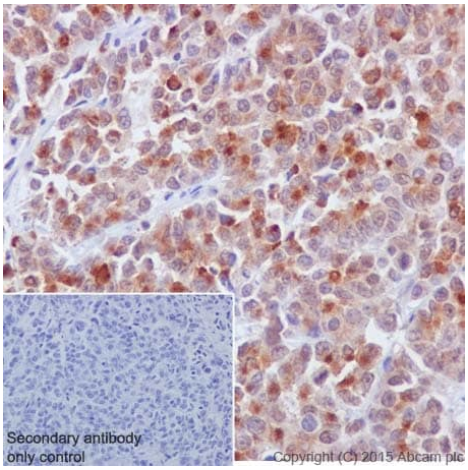
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/1000 dilution

Predicted band size: 82 kDa
Observed band size: 82 kDa

Exposure time: 30 seconds

5% NFDM/TBST: Blocking and diluting buffer.

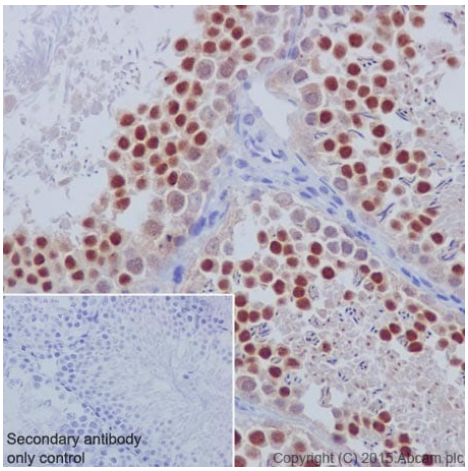


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PI 3 Kinase p85 beta antibody [EPR18416] (ab180967)

Immunohistochemical analysis of paraffin-embedded Human colonic adenocarcinoma tissue labeling PI 3 Kinase p85 beta using ab180967 at 1/100 dilution. A Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) was used as secondary at 1/500 dilution. Nucleus and cytoplasm staining on tumor cells of colonic adenocarcinoma was observed. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

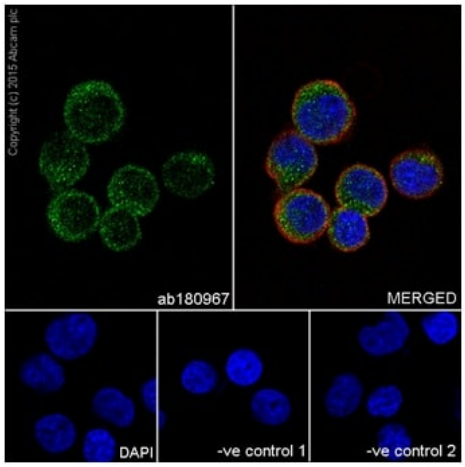


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PI 3 Kinase p85 beta antibody [EPR18416] (ab180967)

Immunohistochemical analysis of paraffin-embedded Rat testis tissue labeling PI 3 Kinase p85 beta using ab180967 at 1/100 dilution. A Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) was used as secondary at 1/500 dilution. Nucleus and cytoplasm staining on spermatogenic cell of rat testis was observed. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



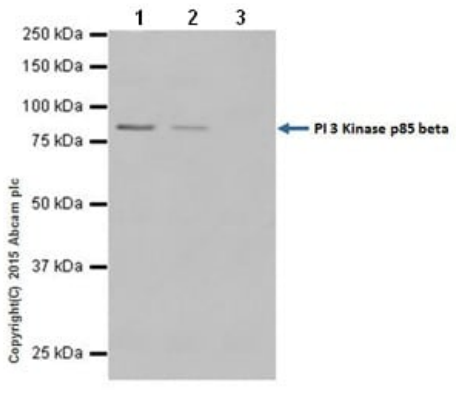
Immunocytochemistry/ Immunofluorescence - Anti-PI 3 Kinase p85 beta antibody [EPR18416] (ab180967)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Jurkat (Human T cell leukemia cells from peripheral blood) cells labeling PI 3 Kinase p85 beta with ab180967 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing nuclear and weakly cytoplasmic staining on Jurkat cell line.

The nuclear counter stain is DAPI (blue).

Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).
The negative controls are as follows:
1. ab180967 at 1/500 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution.
2. **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.



Immunoprecipitation - Anti-PI 3 Kinase p85 beta antibody [EPR18416] (ab180967)

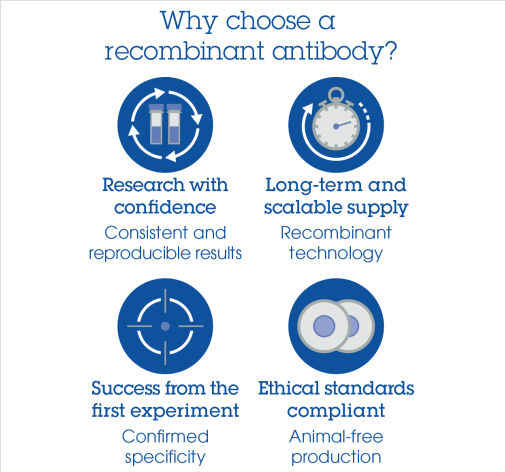
PI 3 Kinase p85 beta was immunoprecipitated from 1mg of HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate with ab180967 at 1/80 dilution. Western blot was performed from the immunoprecipitate using ab180967 at 1/10000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution.

Lane 1: HeLa whole cell lysate10 µg (Input).

Lane 2: ab180967 IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab180967 in HeLa whole cell lysate.
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 10 seconds.



Anti-PI 3 Kinase p85 beta antibody [EPR18416]

(ab180967)

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