abcam

Product datasheet

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



ייבע RabMAb

25 References 画像数 12

製品の概要

製品名 Anti-PI3 Kinase p85 beta antibody [EPR18416]

製品の詳細 Rabbit monoclonal [EPR18416] to PI 3 Kinase p85 beta

由来種 Rabbit

アプリケーション 適用あり: IHC-P, WB, ICC/IF, IP

適用なし: Flow Cyt

種交差性 交差種: Rat, Human

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: 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.

This product is a recombinant monoclonal antibody, which offers several advantages including: 特記事項

- 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**.

製品の特性

製品の状態

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.

Preservative: 0.01% Sodium azide バッファー

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

精製度 Protein A purified

ポリモノ モノクローナル クローン名 **EPR18416**

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab180967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
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.

追加情報

Is unsuitable for Flow Cyt.

ターゲット情報

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Regulatory subunit of phosphoinositide-3-kinase (PI3K), a kinase that phosphorylates Ptdlns(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.

関連疾患

 $\label{lem:megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome 1$

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

翻訳後修飾

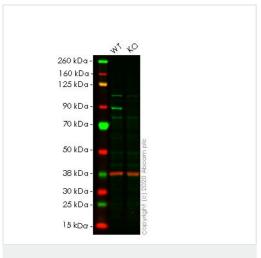
Phosphorylated in response to signaling from activated receptor-type protein kinases (PubMed:19690332, PubMed:20068231). Dephosphorylated by PTPRJ (PubMed:18348712). Dephosphorylated at Tyr-655 by PTPN13. Phosphorylation of Tyr-655 impairs while its

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.



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

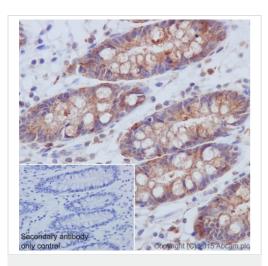
Lysates/proteins at 20 µg per lane.

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 lgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

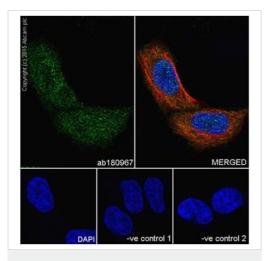


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PI 3 Kinase p85 beta antibody [EPR18416] (ab180967)

Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling PI3 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 lgG 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 PI3 Kinase p85 beta with ab180967 at 1/500 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

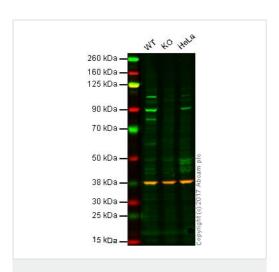
Confocal image showing nuclear and cytoplasmic staining on HeLa cell line.

The nuclear counter stain is DAPI (blue).

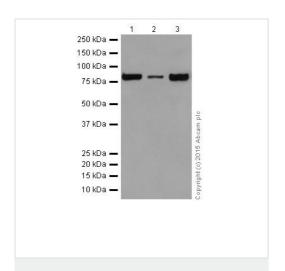
Tubulin is detected with <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution and <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:

ab180967 at 1/500 dilution followed by <u>ab150120</u>
 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution.
 <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/1000 dilution.



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



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, <u>ab8245</u>, 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.

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 lgG 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.

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 lgG (HRP), specific to the non-reduced form of lgG 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.

All lanes : Anti-PI3 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

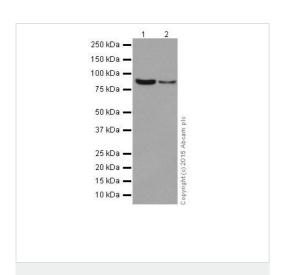
lysate

Lysates/proteins at 10 µg per lane.

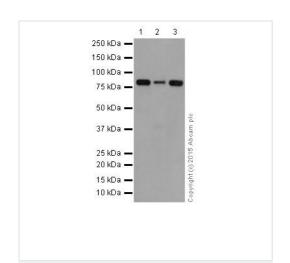
Secondary

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

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



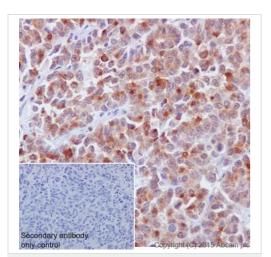
Western blot - Anti-PI 3 Kinase p85 beta antibody [EPR18416] (ab180967)



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

Exposure time: 30 seconds

5% NFDM/TBST: Blocking and diluting buffer.

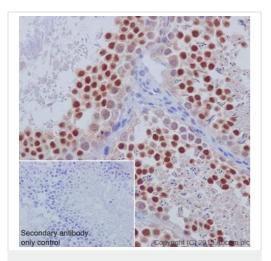


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PI 3 Kinase p85 beta antibody [EPR18416] (ab180967)

Immunohistochemical analysis of paraffin-embedded Human colonic adenocarcinoma tissue labeling PI3 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 lgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

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

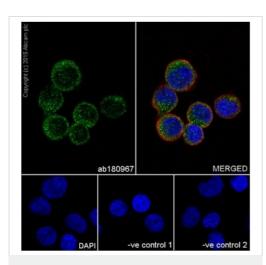


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PI 3 Kinase p85 beta antibody [EPR18416] (ab180967)

Immunohistochemical analysis of paraffin-embedded Rat testis tissue labeling PI3 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 lgG H&L (HRP) (<u>ab97051</u>) 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 lgG (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).

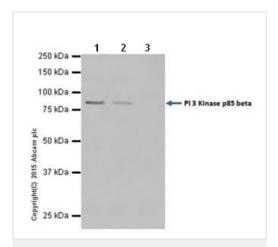
Tubulin is detected with <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution and <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

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. <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG 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 lgG (HRP), specific to the non-reduced form of lgG, was used as secondary antibody at 1/1500 dilution.

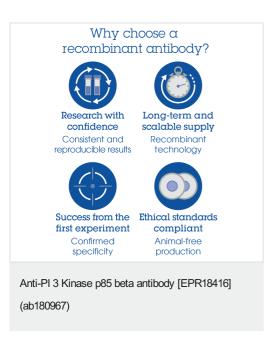
Lane 1: HeLa whole cell lysate10 µg (Input).

Lane 2: ab180967 IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal $\lg G$ ($\underline{ab172730}$) instead of ab180967 in HeLa whole cell lysate.

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

Exposure time: 10 seconds.



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