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Product datasheet

PI3K/AKT signalling pathway panel ab283852

重组

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概述

产品名称	PI3K/AKT signalling pathway组合
种属反应性	**与反应:** Mouse, Rat, Human
产品概述	PI3K/AKT signalling pathway panel ab283852 contains multiple trial-sized versions of antibody clones against PI 3 Kinase p85 alpha, AKT1, AKT2, AKT3 and mTOR specifically selected for high performance in various applications. They are provided as a sampler panel to allow you to easily evaluate each antibody. For guidelines on how to use each antibody within the panel, please consult the individual datasheet for each antibody. Panel contains: - Rabbit monoclonal [EPR18702] to PI 3 Kinase p85 alpha (20 µL) **ab191606** - Rabbit monoclonal [EPR16798] to AKT1 + AKT2 + AKT3 (20 µL) **ab179463** - Rabbit monoclonal [EPR18853] to AKT1 + AKT2 + AKT3 (phospho S472 + S473 + S474) (20 µL) **ab192623** - Rabbit monoclonal [EPR390(N)] to mTOR (20 µL) **ab134903**
说明	Please find more information about PI3K/AKT pathways **here**. We recommend using ab283852 together with **ab205718**. **Explore our range of antibody sample panels** designed to provide you with a variety of trial-size antibodies in a convenient and cost-effective format. Directly conjugated versions of our antibodies are available and ready to use for multicolor flow cytometry or immunocytochemistry analysis. Please refer to the 'Associated products' section below. **Carrier-free formulations** of our recombinant antibodies are also available for easy conjugation to labels of your choice and for multiplex applications. Please refer to the 'Associated products' section below.

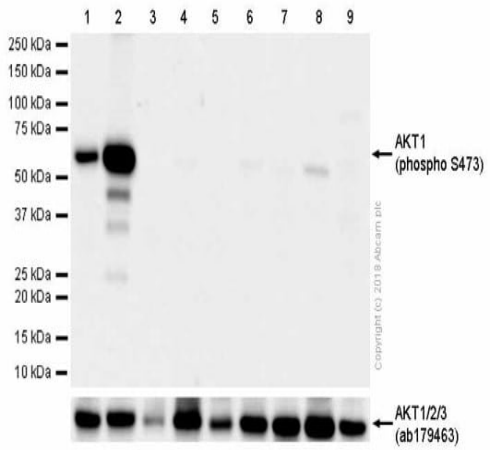
性能

存放说明 Store at -20°C. Please refer to protocols.

组件	1 kit	1 kit
ab192623 - Anti-AKT1 + AKT2 + AKT3 (phospho S472 + S473 + S474) antibody [EPR18853]	2 x 10µl	2 x 10µl
ab134903 - Anti-mTOR antibody [EPR390(N)]	2 x 10µl	2 x 10µl
ab191606 - Anti-PI 3 Kinase p85 alpha antibody [EPR18702]	2 x 10µl	2 x 10µl
ab179463 - Rabbit monoclonal [EPR16798] to AKT1 + AKT2 + AKT3	2 x 10µl	2 x 10µl

细胞定位 mTOR: Endoplasmic reticulum membrane. Golgi apparatus membrane. Mitochondrion outer membrane. Lysosome. Cytoplasm. Nucleus > PML body. Shuttles between cytoplasm and nucleus. Accumulates in the nucleus in response to hypoxia (By similarity). Targeting to lysosomes depends on amino acid availability and RRAGA and RRAGB. AKT1 + AKT2 + AKT3: Cytoplasm. Membrane. Membrane-associated after cell stimulation leading to its translocation.

图片



Western blot - Anti-AKT1 + AKT2 + AKT3 (phospho S472 + S473 + S474) antibody [EPR18853] (

ab192623

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All lanes: Anti-AKT1 + AKT2 + AKT3 (phospho S472 + S473 + S474) antibody [EPR18853] (**ab192623**) at 1/1000 dilution.

Lane 1: LNCaP (Human prostate carcinoma epithelial cell) whole cell lysates, 20 µg

Lane 2: LNCaP (Human prostate carcinoma epithelial cell) treated with 0.1 uM Calyculin A for 30 minutes whole cell lysates, 20 µg

Lane 3: A549 (Human lung carcinoma epithelial cell) whole cell lysates, 20 µg

Lane 4: MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysates, 20 µg

Lane 5: HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysates, 20 µg

Lane 6: HUVEC (Human umbilical vein endothelial cell) whole cell lysates, 20 µg

Lane 7: C2C12 (Mouse myoblasts myoblast) whole cell lysates, 20 µg

Lane 8: Mouse brain lysates, 20 µg

Lane 9: Rat heart lysates, 20 µg

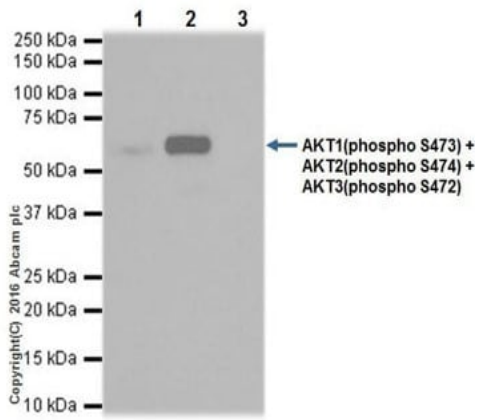
Secondary (all lanes): Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted MW: 56 kDa.

Observed MW: 56 kDa.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 50 seconds.



Immunoprecipitation - Anti-AKT1 + AKT2 + AKT3 (phospho S472 + S473 + S474) antibody [EPR18853] (

ab192623

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KT3 (phospho S472) was immunoprecipitated from 0.35 mg of NIH/3T3 (Mouse embryonic fibroblast cell line) treated with 50ng/ml PDGF for 40min whole cell lysate with **ab192623** at 1/40 dilution. Western blot was performed from the immunoprecipitate using **ab192623** at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1000 dilution.

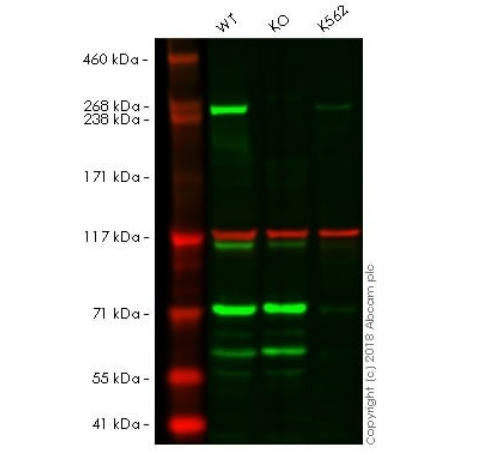
Lane 1: NIH/3T3 treated with 50ng/ml PDGF for 40min whole cell lysate, 10µg (Input).

Lane 2: **ab192623** IP in NIH/3T3 treated with 50ng/ml PDGF for 40min whole cell lysate.

Lane 3: Rabbit IgG,monoclonal [EPR25A]-Isotype Control (**ab172730**) instead of **ab192623** in NIH/3T3 treated with 50ng/ml PDGF for 40min whole cell lysate.

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

Exposure time: 5 seconds.



Western blot - Anti-mTOR antibody [EPR390(N)] (

ab134903

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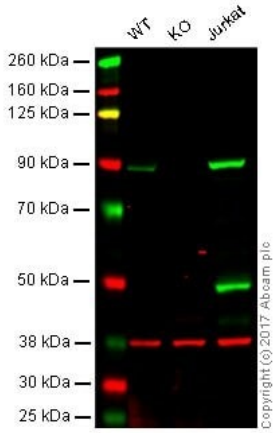
Lanes 1 - 3: Merged signal (red and green). Green - **ab134903** observed at 289 kDa. Red - loading control, **ab130007**, observed at 130 kDa.

Lane 1: Wild-type HEK293T whole cell lysate, 20 µg

Lane 2: MTOR knockout HEK293T whole cell lysate, 20 µg

Lane 3: K562 whole cell lysate, 20 µg

ab134903 was shown to specifically react with mTOR in wild-type HEK293T cells as signal was lost in MTOR knockout cells. Wild-type and MTOR knockout samples were subjected to SDS-PAGE. Ab134903 and **ab130007** (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/10000 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.



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

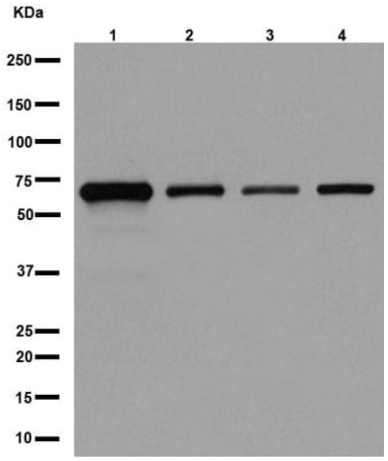
ab191606

)

Lanes 1 - 3: Merged signal (red and green). Green - **ab191606** observed at 90 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

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

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.



Western blot - Anti-AKT1 + AKT2 + AKT3 antibody [EPR16798] (

ab179463

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All lanes: Anti-AKT1 + AKT2 + AKT3 antibody [EPR16798] (**ab179463**) at 1/1000 dilution.

Lane 1: MCF7 (Human breast adenocarcinoma cell line) whole cell lysates, 20 µg

Lane 2: HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysates, 20 µg

Lane 3: Hep G2 (Human liver hepatocellular carcinoma) whole cell lysates, 20 µg

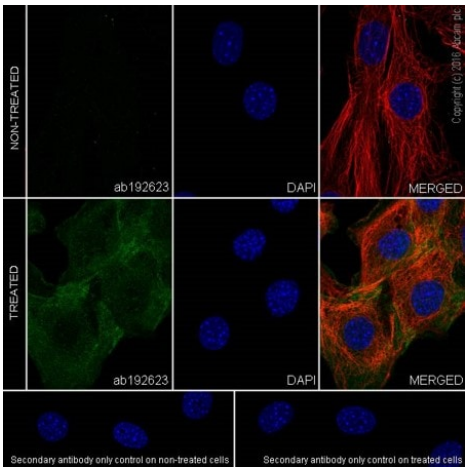
Secondary (all lanes): Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution.

Predicted MW: 56 kDa.

Observed MW: 56 kDa.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 70 seconds.



Immunocytochemistry - Anti-AKT1 + AKT2 + AKT3 (phospho S472 + S473 + S474) antibody [EPR18853] (

ab192623

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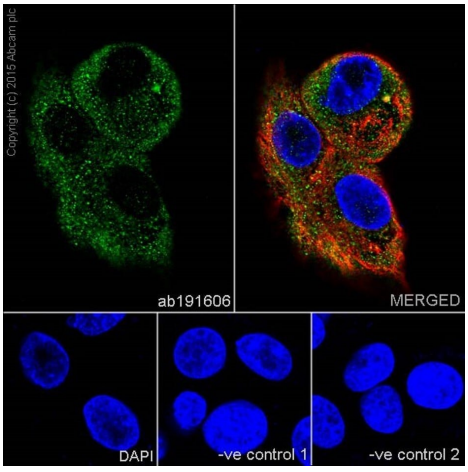
Immunocytochemical analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embryonic fibroblast cell line) cells, untreated or treated with PDGF (100 ng/ml) for 1 hour, labeling AKT3 (phospho S472) + AKT2 (phospho S474) + AKT1 (phospho S473) with **ab192623** at 1/100 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

The signal increased after treatment with PDGF (100 ng/ml) for 1 hour on NIH/3T3 cells.

The nuclear counter stain is DAPI (blue).

Tubulin is detected with **ab195889** (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution.



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

ab191606

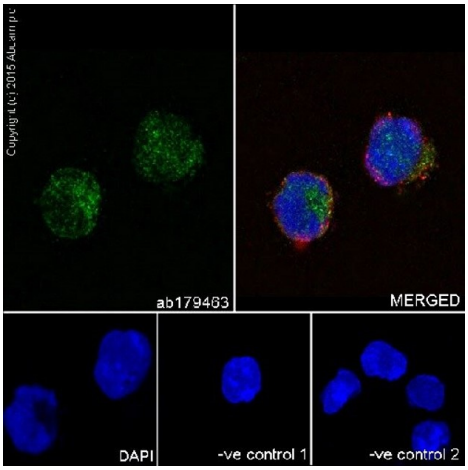
)

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.



Immunocytochemistry - Anti-AKT1 + AKT2 + AKT3 antibody [EPR16798] (

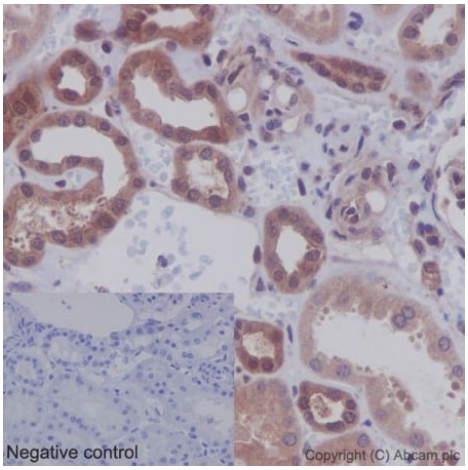
ab179463

)

Immunocytochemical analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized K562 (Human chronic myelogenous leukemia cells from bone marrow) cells labeling AKT1 + AKT2 + AKT3 with **ab179463** at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/400 dilution (green). Cytoplasm and nuclear staining on K562 cell line is observed. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:

1. **ab179463** at 1/100 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
2. **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/400 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AKT1 + AKT2 + AKT3 antibody [EPR16798] (

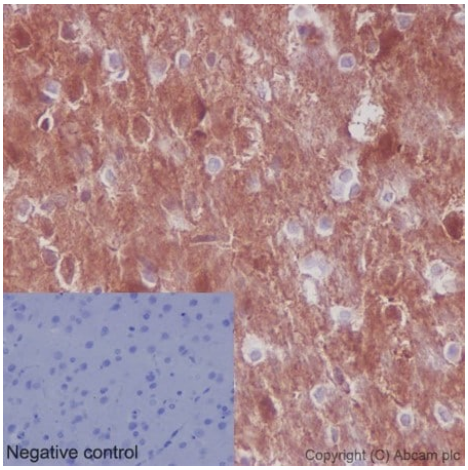
ab179463

)

Immunohistochemical analysis of paraffin-embedded Human kidney tissue labeling AKT1 + AKT2 + AKT3 with **ab179463** at 1/250 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Cytoplasm and nucleus staining on Human renal cortex is observed. Counterstained with Hematoxylin.

Negative control: Using PBS instead of primary ab, secondary ab is prediluted HRP Polymer for Rabbit/Mouse IgG.

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-AKT1 + AKT2 + AKT3 antibody [EPR16798] (

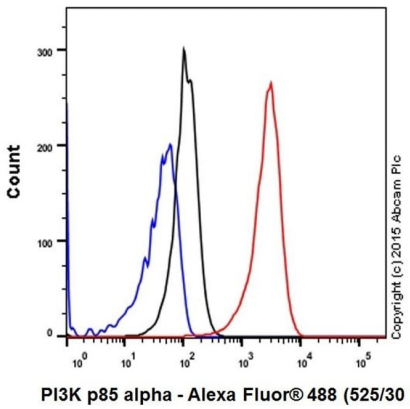
ab179463

)

Immunohistochemical analysis of paraffin-embedded Rat cerebral cortex tissue labeling AKT1 + AKT2 + AKT3 with **ab179463** at 1/250 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Cytoplasm and nucleus staining on Rat cerebral cortex is observed. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary ab, secondary ab is prediluted HRP Polymer for Rabbit/Mouse IgG.

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

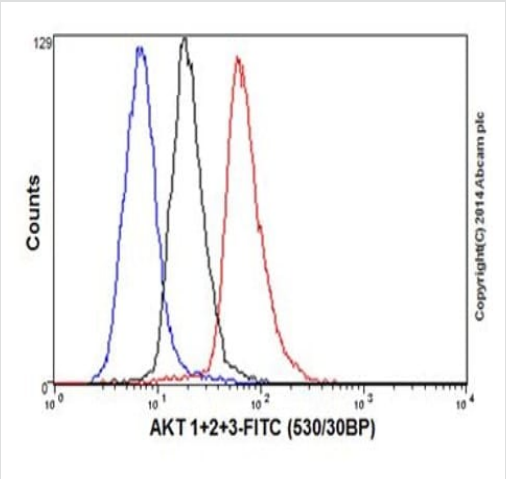


Flow Cytometry (Intracellular) - Anti-PI 3 Kinase p85 alpha antibody [EPR18702] (

ab191606

)

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.



Flow Cytometry (Intracellular) - Anti-AKT1 + AKT2 + AKT3 antibody [EPR16798] (

ab179463

)

Flow cytometric analysis of 2% paraformaldehyde-fixed A549 (Human lung carcinoma) cells labeling AKT1 + AKT2 + AKT3 with **ab179463** at 1/330 dilution (red) compared with a rabbit monoclonal IgG isotype control (black) and a unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/150 dilution was used as the secondary antibody.

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