abcam

Product datasheet

Anti-AKT1 (pS473) + AKT2 (pS474) + AKT3 (pS472) antibody [EPR18853] ab192623

重组 RabMAb

53 References 7 图像

概述

产品名称	Anti-AKT1 (pS473) + AKT2 (pS474) + AKT3 (pS472)**抗体**[EPR18853]
描述	**兔单克隆抗体**[EPR18853] to AKT3 (phospho S472) + AKT2 (phospho S474) + AKT1 (phospho S473)
宿主	Rabbit
经测试应用	**适用于:** ICC/IF, WB, IP, Dot blot
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: MCF7 whole cell lysate treated with 100ng/ml IGF-1 for 15 minutes; PC-12 and NIH/3T3 whole cell lysates treated with 100ng/ml PDGF for 60 minutes. ICC/IF: NIH/3T3 cells treated with PDGF (100 ng/ml) for 1 hour. IP: NIH/3T3 treated with 50ng/ml PDGF for 40min whole 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	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified

克隆	单克隆
克隆编号	EPR18853
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab192623于以下的经测试应用**

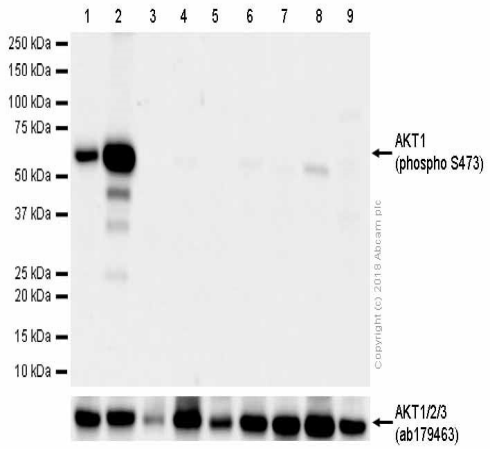
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ICC/IF		1/100.
WB		1/1000. Detects a band of approximately 56 kDa (predicted molecular weight: 56 kDa).
IP		1/40.
Dot blot		1/1000.

靶标

细胞定位 AKT3: Cytoplasm. Membrane. Membrane-associated after cell stimulation leading to its translocation. AKT1: Cytoplasm. Nucleus. Cell membrane. Nucleus after activation by integrin-linked protein kinase 1 (ILK1). Nuclear translocation is enhanced by interaction with TCL1A. Phosphorylation on Tyr-176 by TNK2 results in its localization to the cell membrane where it is targeted for further phosphorylations on Thr-308 and Ser-473 leading to its activation and the activated form translocates to the nucleus.

图片



Western blot - Anti-AKT1 (pS473) + AKT2 (pS474) + AKT3 (pS472) antibody [EPR18853] (ab192623)

All lanes : Anti-AKT1 (pS473) + AKT2 (pS474) + AKT3 (pS472) antibody [EPR18853] (ab192623) at 1/1000 dilution

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

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

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

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

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

Lane 6 : HUVEC (Human umbilical vein endothelial cell) whole cell

lysates

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

Lane 8 : Mouse brain lysates

Lane 9 : Rat heart lysates

Lysates/proteins at 20 µg per lane.

Secondary

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

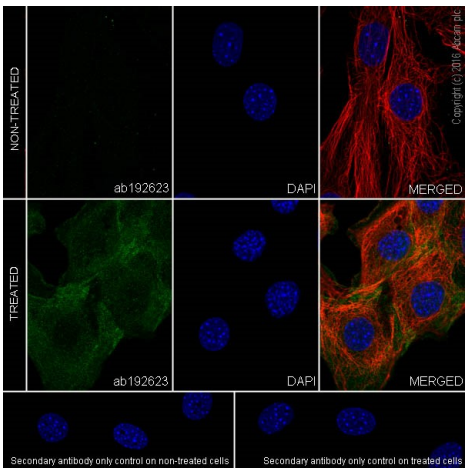
Predicted band size: 56 kDa

Observed band size: 56 kDa

Exposure time: 50 seconds

The basal expression level of AKT1 (phospho S473) varies in different cell lines reported by PMID: 19372546. But to detect clear signal, treatment is strongly recommended when using this antibody.

Blocking and Diluting buffer: 5% NFDM/TBST



Immunocytochemistry/ Immunofluorescence - Anti-AKT1 (pS473) + AKT2 (pS474) + AKT3 (pS472) antibody [EPR18853] (ab192623)

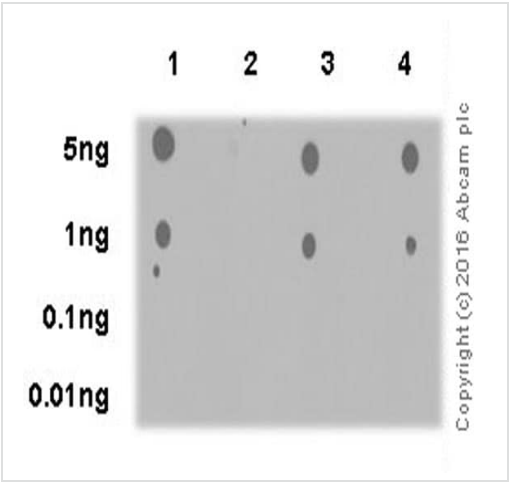
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embryonic fibroblast cell line) cells, untreated or treated with PDGF (100 ng/ml) for 1 hour, labeling AKT3 (phospho S472) + AKT2 (phospho S474) + AKT1 (phospho S473) with ab192623 at 1/100 dilution, 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.



Dot Blot - Anti-AKT1 (pS473) + AKT2 (pS474) + AKT3 (pS472) antibody [EPR18853] (ab192623)

Dot blot analysis of AKT3 (phospho S472) labeled with ab192623 at 1/1000 dilution.

Lane 1: AKT3 (phospho S472) phospho peptide;

Lane 2: AKT3 non-phospho peptide;

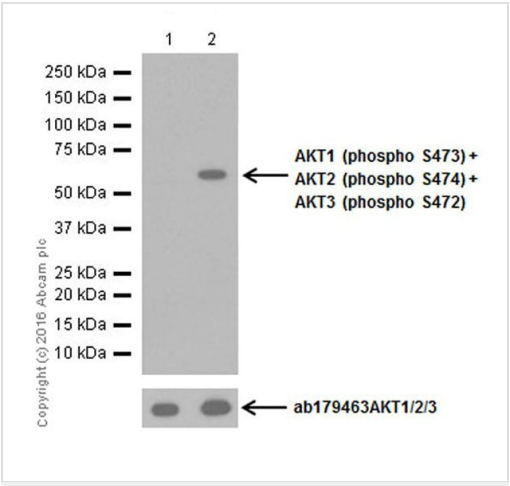
Lane 3: AKT1 (phospho S473) phospho peptide;

Lane 4: AKT2 (phospho S474) phospho peptide.

Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated (**ab97051**) at 1/100000 dilution was used as secondary antibody.

Blocking and diluting buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



Western blot - Anti-AKT1 (pS473) + AKT2 (pS474) + AKT3 (pS472) antibody [EPR18853] (ab192623)

All lanes : Anti-AKT1 (pS473) + AKT2 (pS474) + AKT3 (pS472) antibody [EPR18853] (ab192623) at 1/1000 dilution

Lane 1 : Untreated MCF7 (Human breast adenocarcinoma cell line) whole cell lysate
Lane 2 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate treated with 100ng/ml IGF-1 for 15 minutes

Lysates/proteins at 10 µg per lane.

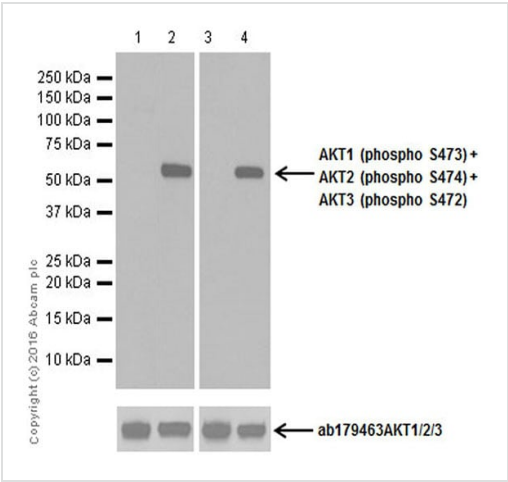
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 56 kDa
Observed band size: 56 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

Phosphorylation of AKT at S473 can be induced by IGF-1 treatment according to the literature (PMID: 23638184).



Western blot - Anti-AKT1 (pS473) + AKT2 (pS474) + AKT3 (pS472) antibody [EPR18853] (ab192623)

All lanes : Anti-AKT1 (pS473) + AKT2 (pS474) + AKT3 (pS472) antibody [EPR18853] (ab192623) at 1/1000 dilution

Lane 1 : Untreated PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate
Lane 2 : PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate treated with 100ng/ml PDGF for 60 minutes
Lane 3 : Untreated NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate
Lane 4 : NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate treated with 100ng/ml PDGF for 60 minutes

Lysates/proteins at 20 µg per lane.

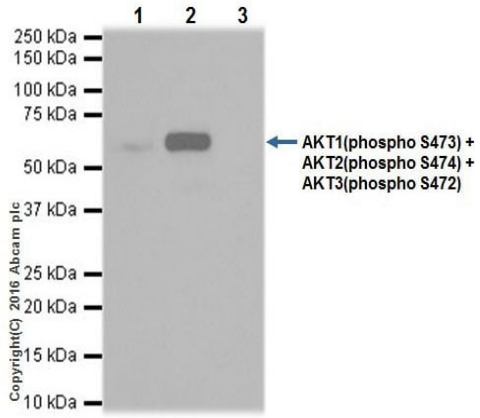
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 56 kDa
Observed band size: 56 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1 and 2: 15 seconds; Lane 3 and 4: 3 minutes.

Phosphorylation of AKT can be induced by PDGF treatment according to the literature (PMID: 10984605 and 7774014).



Immunoprecipitation - Anti-AKT1 (pS473) + AKT2 (pS474) + AKT3 (pS472) antibody [EPR18853] (ab192623)

AKT3 (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.

Anti-AKT1 (pS473) + AKT2 (pS474) + AKT3 (pS472) antibody [EPR18853] (ab192623)

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours

- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.cn/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors