

Product datasheet

Anti-AKT1 + AKT2 + AKT3 (phospho S472 + S473 + S474) antibody [EPR18853] ab192623

Recombinant RabMAb

[53 References](#) [7 Images](#)

Overview

Product name	Anti-AKT1 + AKT2 + AKT3 (phospho S472 + S473 + S474) antibody [EPR18853]
Description	Rabbit monoclonal [EPR18853] to AKT1 + AKT2 + AKT3 (phospho S472 + S473 + S474)
Host species	Rabbit
Tested applications	Suitable for: ICC/IF, WB, IP, Dot blot
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	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.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none">- High batch-to-batch consistency and reproducibility- Improved sensitivity and specificity- Long-term security of supply- Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

Properties

Form	Liquid
Storage instructions	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.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number EPR18853
Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab192623 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
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.

Target

Function IGF-1 leads to the activation of AKT3, which may play a role in regulating cell survival. Capable of phosphorylating several known proteins. Truncated isoform 2/PKB gamma 1 without the second serine phosphorylation site could still be stimulated but to a lesser extent.

Tissue specificity In adult tissues, it is highly expressed in brain, lung and kidney, but weakly in heart, testis and liver. In fetal tissues, it is highly expressed in heart, liver and brain and not at all in kidney.

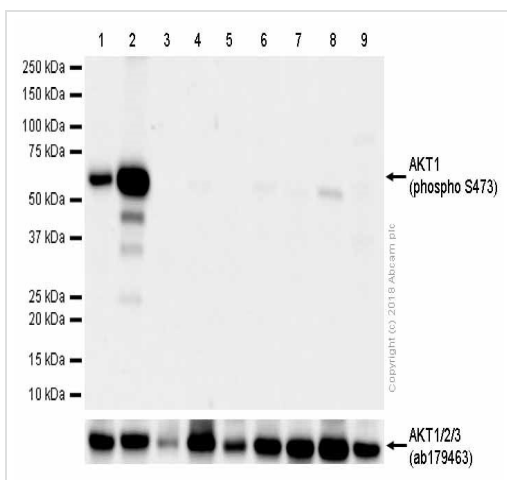
Sequence similarities Belongs to the protein kinase superfamily. AGC Ser/Thr protein kinase family. RAC subfamily. Contains 1 AGC-kinase C-terminal domain. Contains 1 PH domain. Contains 1 protein kinase domain.

Domain Binding of the PH domain to the phosphatidylinositol 3-kinase alpha (PI(3)K) results in its targeting to the plasma membrane.

Post-translational modifications Phosphorylation on Thr-305 and Ser-472 is required for full activity (By similarity). Phosphorylated upon DNA damage, probably by ATM or ATR. Ubiquitinated. When fully phosphorylated and translocated into the nucleus, undergoes 'Lys-48'-polyubiquitination catalyzed by TTC3, leading to its degradation by the proteasome.

Cellular localization Cytoplasm. Membrane. Membrane-associated after cell stimulation leading to its translocation.

Images



Western blot - Anti-AKT1 + AKT2 + AKT3 (phospho S472 + S473 + S474) antibody [EPR18853] (ab192623)

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

Lane 2 : LNCaP (Human prostate carcinoma epithelial cell) treated with 0.1 μ M 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 μ g per lane.

Secondary

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

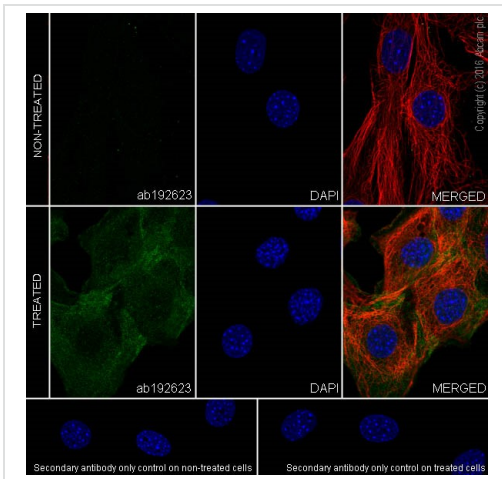
Predicted band size: 56 kDa

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 + AKT2 + AKT3 (phospho S472 + S473 + S474) antibody [EPR18853] (ab192623)

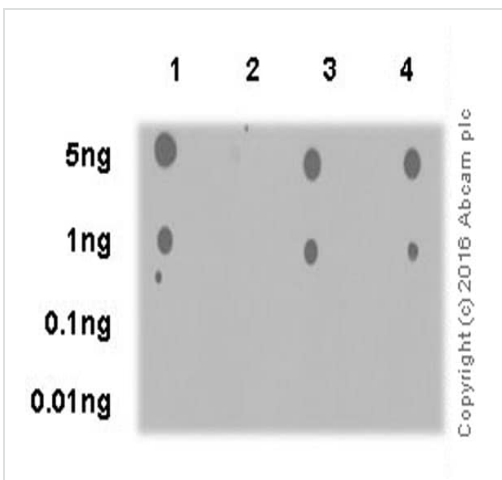
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embryonic fibroblast cell line) cells, untreated or treated with PDGF (100 ng/ml) for 1 hour, labeling AKT3 (phospho S472) + AKT2 (phospho S474) + AKT1 (phospho S473) with ab192623 at 1/100 dilution,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

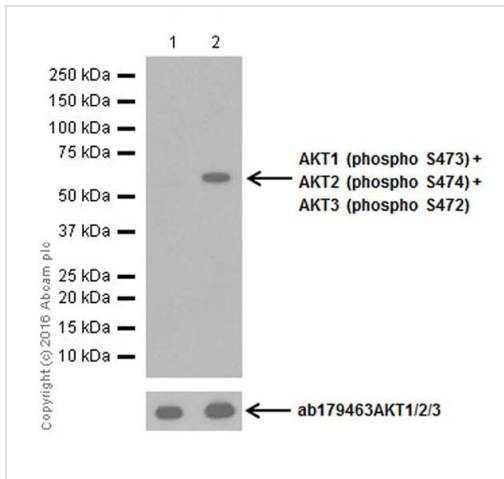
Lane 3: AKT1 (phospho S473) phospho peptide;

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 + AKT2 + AKT3 (phospho S472 + S473 + S474) antibody [EPR18853] (ab192623)

All lanes : Anti-AKT1 + AKT2 + AKT3 (phospho S472 + S473 + S474) 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

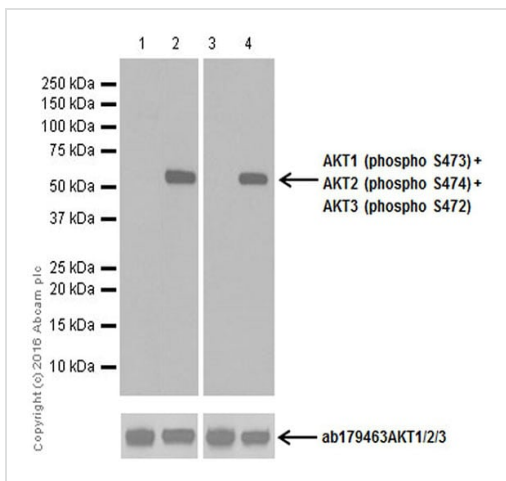
Predicted band size: 56 kDa

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 + AKT2 + AKT3 (phospho S472 + S473 + S474) antibody [EPR18853] (ab192623)

All lanes : Anti-AKT1 + AKT2 + AKT3 (phospho S472 + S473 + S474) 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

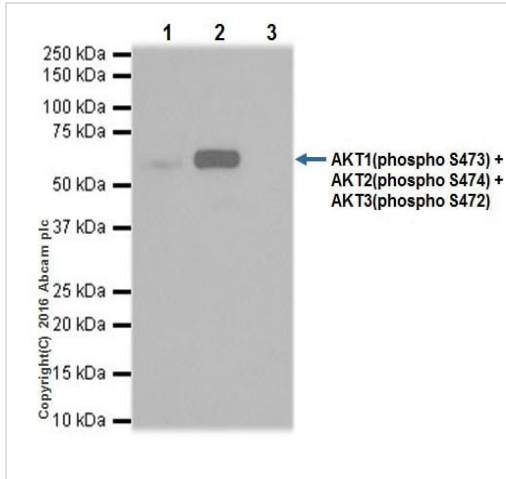
Predicted band size: 56 kDa

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 + AKT2 + AKT3 (phospho S472 + S473 + S474) 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.

Why choose a recombinant antibody?

<p>Research with confidence Consistent and reproducible results</p>	<p>Long-term and scalable supply Recombinant technology</p>
<p>Success from the first experiment Confirmed specificity</p>	<p>Ethical standards compliant Animal-free production</p>

Anti-AKT1 + AKT2 + AKT3 (phospho S472 + S473 + S474) antibody [EPR18853] (ab192623)

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