

Product datasheet

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

Recombinant RabMAb

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

Overview

Product name	Anti-AKT1 (pS473) + AKT2 (pS474) + AKT3 (pS472) antibody [EPR18853]
Description	Rabbit monoclonal [EPR18853] to AKT3 (phospho S472) + AKT2 (phospho S474) + AKT1 (phospho S473)
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.

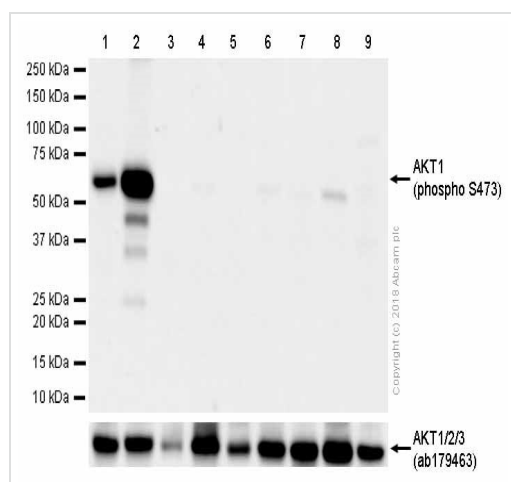
Application	Abreviews	Notes
ICC/IF		1/100.
WB		1/1000. Detects a band of approximately 56 kDa (predicted molecular weight: 56 kDa).
IP		1/40.
Dot blot		1/1000.

Target

Cellular localization

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.

Images



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 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 µg per lane.

Secondary

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

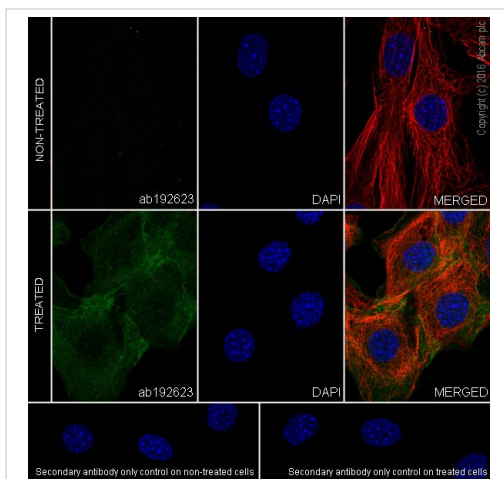
Predicted band size: 56 kDa

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](#))

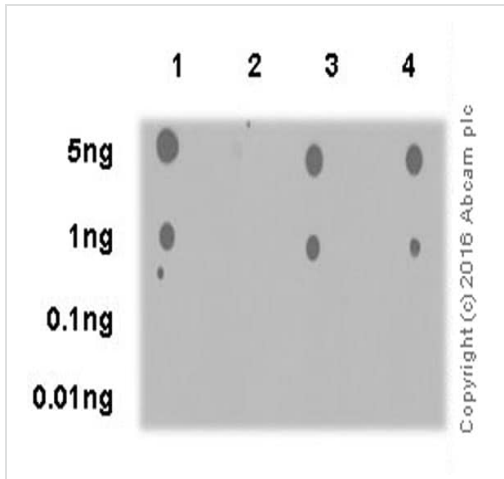
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embryonic fibroblast cell line) cells, untreated or treated with PDGF (100 ng/ml) for 1 hour, labeling AKT3 (phospho S472) + AKT2 (phospho S474) + AKT1 (phospho S473) with [ab192623](#) at 1/100 dilution,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

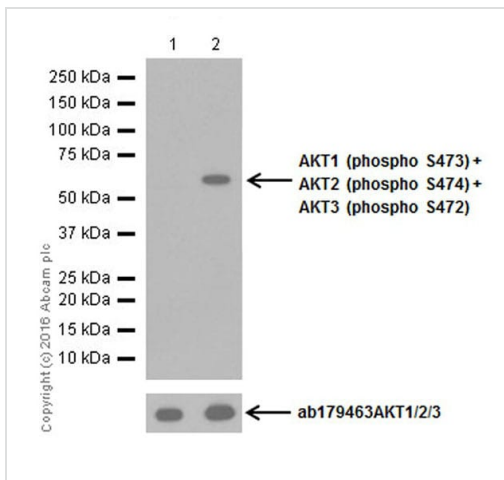
Lane 3: AKT1 (phospho S473) phospho peptide;

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% NFD/MTBST.

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

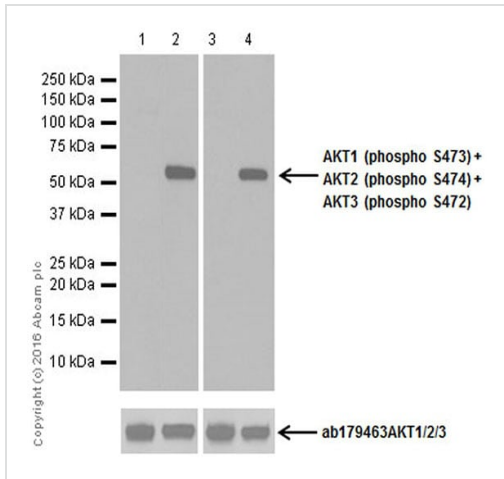
Predicted band size: 56 kDa

Observed band size: 56 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFD/MTBST.

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

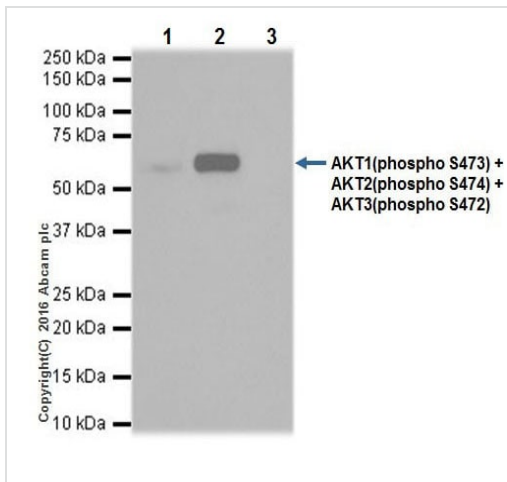
Predicted band size: 56 kDa

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.

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

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