abcam

Product datasheet

Anti-AKT1 (pS473) + AKT2 (pS474) + AKT3 (pS472) antibody [EPR18853] - BSA and Azide free ab222489

Recombinant RabMAb

★★★★★ 1 Abreviews 2 References 4 Images

Overview

Product name	Anti-AKT1 (pS473) + AKT2 (pS474) + AKT3 (pS472) antibody [EPR18853] - BSA and Azide free
Description	Rabbit monoclonal [EPR18853] to AKT3 (phospho S472) + AKT2 (phospho S474) + AKT1 (phospho S473) - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, 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	ab222489 is the carrier-free version of **ab192623**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR18853
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab222489 in the following tested applications.

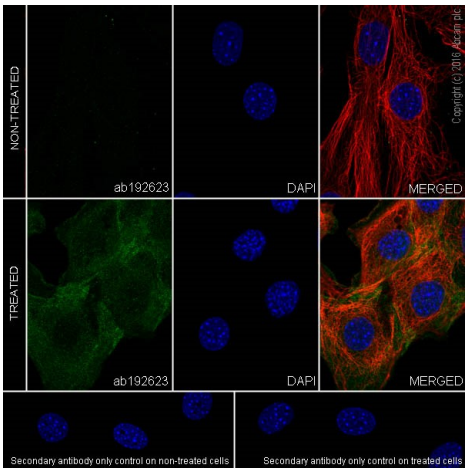
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★★ **(1)**	Use at an assay dependent concentration. Detects a band of approximately 56 kDa (predicted molecular weight: 56 kDa).
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
Dot blot		Use at an assay dependent concentration.

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



Immunocytochemistry/ Immunofluorescence - Anti-AKT1 (pS473) + AKT2 (pS474) + AKT3 (pS472) antibody [EPR18853] - BSA and Azide free (ab222489)

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).

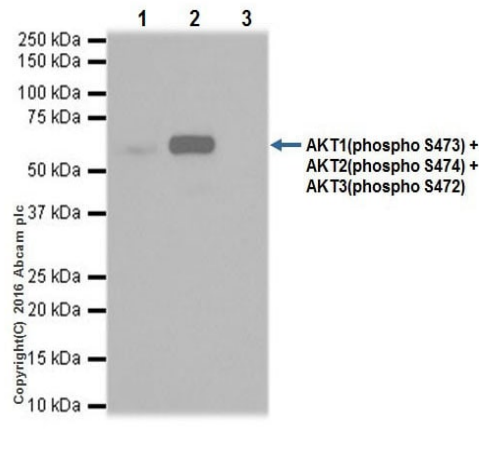
The signal increased after treatment with PDGF (100 ng/ml) for 1 hour on NIH/3T3 cells.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab192623**).



Immunoprecipitation - Anti-AKT1 (pS473) + AKT2 (pS474) + AKT3 (pS472) antibody [EPR18853] - BSA and Azide free (ab222489)

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).

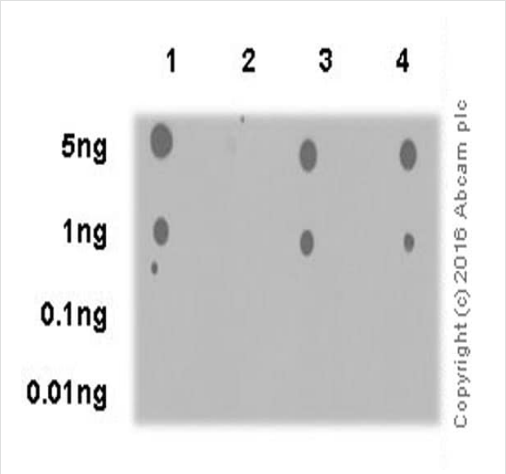
Lane 2: **ab192623** IP in NIH/3T3 treated with 50ng/ml PDGF for 40min whole cell lysate.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab192623**).



Dot Blot - Anti-AKT1 (pS473) + AKT2 (pS474) + AKT3 (pS472) antibody [EPR18853] - BSA and Azide free (ab222489)

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;

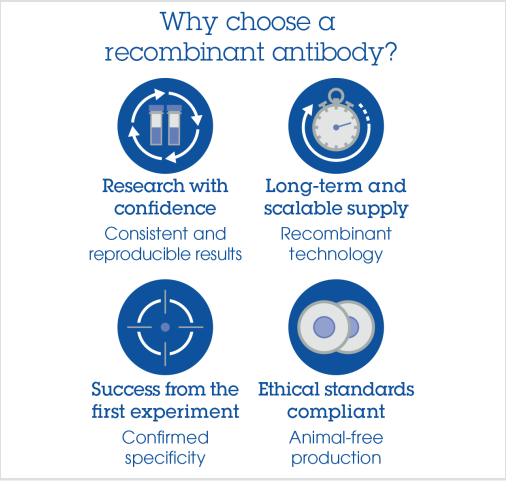
Lane 4: AKT2 (phospho S474) phospho peptide.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab192623**).



Anti-AKT1 (pS473) + AKT2 (pS474) + AKT3 (pS472) antibody [EPR18853] - BSA and Azide free (ab222489)

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.com/abpromise** or contact our technical team.

Terms and conditions

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