# abcam

## Product datasheet

## Anti-AKT3 + AKT2 + AKT1 antibody [Y89] - BSA and Azide free ab219588

יובעדער RabMAb

26 References 画像数 5

## 製品の概要

免疫原

特記事項

製品名 Anti-AKT3 + AKT2 + AKT1 antibody [Y89] - BSA and Azide free

製品の詳細 Rabbit monoclonal [Y89] to AKT3 + AKT2 + AKT1 - BSA and Azide free

由来種 Rabbit

特異性 This product reacts with AKT1, AKT2 and AKT3.

アプリケーション 適用あり: Flow Cyt (Intra), ICC/IF, WB, IHC-P, IP

種交差性 交差種: Human

交差が予測される動物種: Mouse, Rat, Cow 🕰

Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール MCF7 cell lysate and prostate carcinoma tissue. IP: MCF7 cell lysate

ab219588 is the carrier-free version of ab32505.

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 cellbased 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<sup>®</sup> 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

## 製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C. Do Not Freeze.

**バッファー** pH: 7.20

Constituent: PBS

キャリア・フリー はい

精製度 Protein A purified

**ポリ/モノ** モノクローナル

**クローン名** Y89 アイソタイプ IgG

## アプリケーション

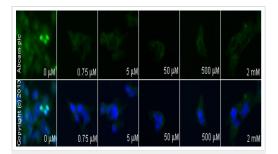
**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab219588の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		Use at an assay dependent concentration. <b>ab199376</b> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 59 kDa (predicted molecular weight: 56 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP		Use at an assay dependent concentration.

#### ターゲット情報

#### 細胞内局在

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.

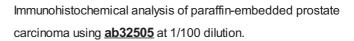


Immunocytochemistry/ Immunofluorescence - Anti-AKT3 + AKT2 + AKT1 antibody [Y89] - BSA and Azide free (ab219588)

<u>ab32505</u> staining in SK-N-SH cells treated with alsterpaullone (<u>ab141070</u>), by ICC/IF. Decrease of AKT1 + AKT2 + AKT3 expression correlates with increased concentration of alsterpaullone, as described in literature.

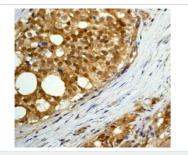
The cells were incubated at 37°C for 6h in media containing different concentrations of <u>ab141070</u> (alsterpaullone) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with <u>ab32505</u> (1/200 dilution was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 anti-rabbit polyclonal antibody (<u>ab96899</u>) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab32505</u>).

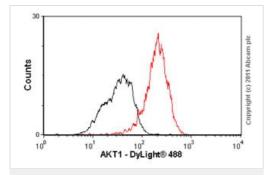


This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32505).

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



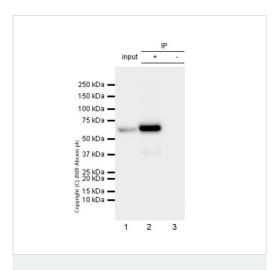
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-AKT3 + AKT2 + AKT1 antibody [Y89] - BSA and Azide free (ab219588)



Flow Cytometry (Intracellular) - Anti-AKT3 + AKT2 + AKT1 antibody [Y89] - BSA and Azide free (ab219588)

Overlay histogram showing HeLa cells stained with <u>ab32505</u> (red line). The cells were fixed with methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (<u>ab32505</u>, 1/20 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit lgG (H+L) (<u>ab96899</u>) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit monoclonal lgG (1µg/1x10<sup>6</sup> cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a slightly decreased signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized in 0.1% PBS-Tween used under the same conditions. This data

was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32505).



Immunoprecipitation - Anti-AKT3 + AKT2 + AKT1 antibody [Y89] - BSA and Azide free (ab219588)

Purified  $\underline{ab32505}$  at 1/50 dilution (2µg) immunoprecipitating AKT3+AKT2+AKT1 in MCF7 whole cell lysate.

Lane 1 (input): MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate 10µg

Lane 2 (+): <u>ab32505</u> + MCF7 whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (  $\underline{ab172730}$  ) instead of  $\underline{ab32505}$  in MCF7 whole cell lysate.

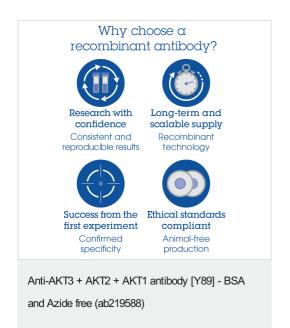
VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) (1/1000 dilution) was used for Western blotting.

Blocking Buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.

Observed band size: 59 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32505).



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