abcam

Product datasheet

Anti-AKT3 + AKT2 + AKT1 antibody [Y89] ab32505

Recombinant RabMAb

★★★★★ 6 Abreviews 139 References 8 Images

Overview

Product name	Anti-AKT3 + AKT2 + AKT1 antibody [Y89]
Description	Rabbit monoclonal [Y89] to AKT3 + AKT2 + AKT1
Host species	Rabbit
Specificity	This product reacts with AKT1, AKT2 and AKT3.
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, WB, IHC-P, IP
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat, Cow
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	MCF7 cell lysate and prostate carcinoma tissue. IP: MCF7 cell lysate
General notes	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. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	Y89
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32505 in the following tested applications.

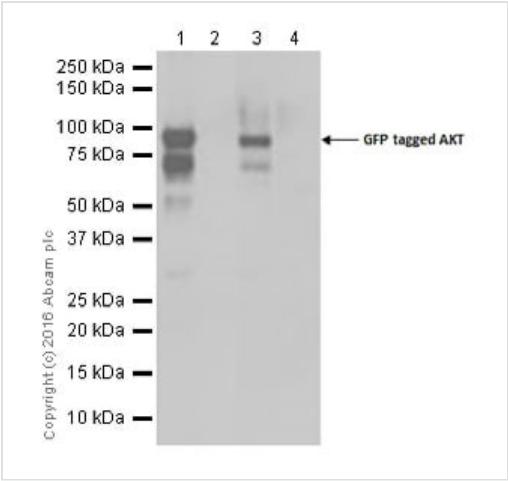
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/20. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/100 - 1/250.
WB	★★★★★ (3)	1/2000 - 1/10000. Detects a band of approximately 59 kDa (predicted molecular weight: 56 kDa).
IHC-P	★★★★☆ (1)	1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP		1/50.

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-AKT3 + AKT2 + AKT1 antibody [Y89] (ab32505)

All lanes : Anti-AKT3 + AKT2 + AKT1 antibody [Y89] (ab32505) at 1/10000 dilution

Lane 1 : 293T cell lysate transfected with GFP tagged AKT1
Lanes 2 & 4 : 293T cell lysate transfected with empty vector
Lane 3 : 293T cell lysate transfected with GFP tagged AKT3

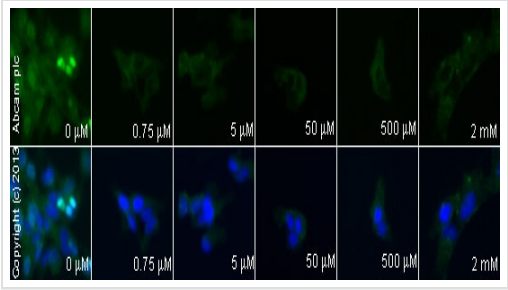
Lysates/proteins at 10 µg per lane.

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

Predicted band size: 56 kDa
Observed band size: 82 kDa

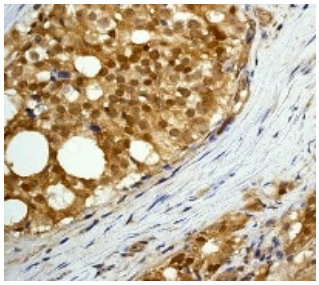
Exposure time: 8 seconds

Blocking and diluting buffer and concentration: 5% NFDM/TBST



Immunocytochemistry/ Immunofluorescence - Anti-AKT3 + AKT2 + AKT1 antibody [Y89] (ab32505)

ab32505 staining in SK-N-SH cells treated with alsterpaullone (**ab141070**), 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 **ab141070** (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 ab32505 (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 (**ab96899**) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.



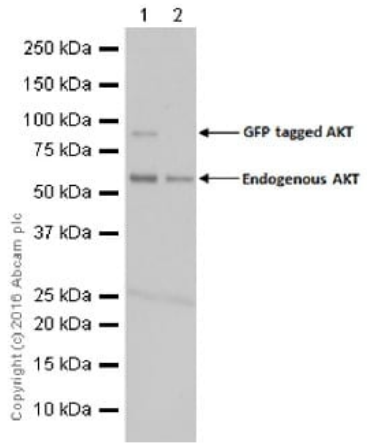
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AKT3 + AKT2 + AKT1 antibody [Y89] (ab32505)

Immunohistochemical analysis of paraffin-embedded prostate carcinoma using ab32505 at 1/100 dilution.

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

Immunoprecipitation - Anti-AKT3 + AKT2 + AKT1 antibody [Y89] (ab32505)

Purified 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 (+): ab32505 + MCF7 whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32505 in MCF7 whole cell lysate.
VeriBlot for IP Detection Reagent (HRP) (**ab131366**) (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



Western blot - Anti-AKT3 + AKT2 + AKT1 antibody [Y89] (ab32505)

All lanes : Anti-AKT3 + AKT2 + AKT1 antibody [Y89] (ab32505) at 1/2000 dilution

Lane 1 : 293T cell lysate transfected with GFP tagged AKT2
Lane 2 : 293T cell lysate transfected with empty vector

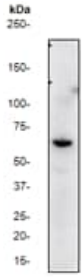
Lysates/proteins at 10 µg per lane.

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

Predicted band size: 56 kDa
Observed band size: 82 kDa

Exposure time: 5 seconds

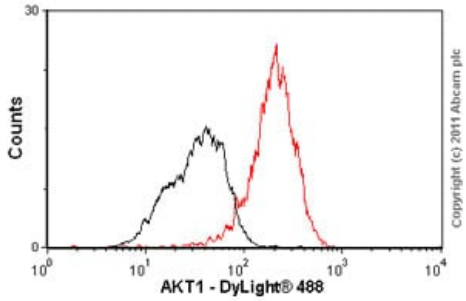
Blocking and diluting buffer and concentration: 5% NFDM/TBST



Western blot - Anti-AKT3 + AKT2 + AKT1 antibody [Y89] (ab32505)

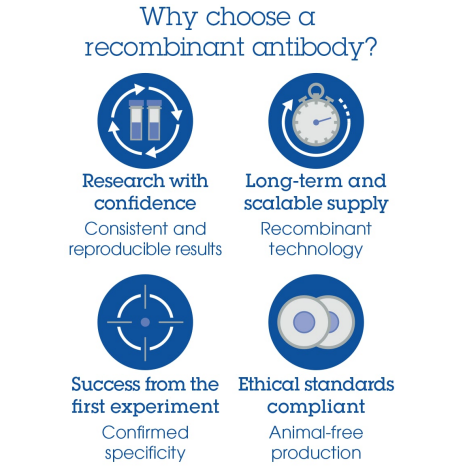
Anti-AKT3 + AKT2 + AKT1 antibody [Y89] (ab32505) at 1/10000 dilution + MCF-7 cell lysate

Predicted band size: 56 kDa
Observed band size: 59 kDa



Flow Cytometry (Intracellular) - Anti-AKT3 + AKT2 + AKT1 antibody [Y89] (ab32505)

Overlay histogram showing HeLa cells stained with ab32505 (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 (ab32505, 1/20 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit monoclonal IgG (1µg/1x10^6 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.



Anti-AKT3 + AKT2 + AKT1 antibody [Y89] (ab32505)

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