Product datasheet

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

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: ICC/IF, WB, IHC-P, Flow Cyt, IP
Species reactivity Reacts with: Mouse, Human
Predicted to work with: Rat, Cow
Immunogen          Synthetic peptide within Human AKT1 aa 450 to the C-terminus (C terminal). The exact sequence is proprietary.
Database link: P31749
Positive control   MCF7 cell lysate and prostate carcinoma tissue.
General notes      A trial size is available to purchase for this antibody.

Our RabMab® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents
This product is a recombinant rabbit monoclonal antibody.

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, 0.05% BSA
Purity            Protein A purified
Clonality         Monoclonal
Clone number      Y89
Isotype           IgG
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.

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.

Applications

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.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>ICC/IF</td>
<td>1/100 - 1/250.</td>
<td></td>
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<tr>
<td>WB</td>
<td>🌟🌟🌟🌟 1/2000 - 1/10000. Detects a band of approximately 59 kDa (predicted molecular weight: 56 kDa).</td>
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<tr>
<td>IHC-P</td>
<td>🌟🌟🌟      1/100.</td>
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<tr>
<td>Flow Cyt</td>
<td>1/20. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
<td></td>
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<tr>
<td>IP</td>
<td>1/100.</td>
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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-AKT3 + AKT2 + AKT1 antibody [Y89] (ab32505)
Immunohistochemical analysis of paraffin-embedded prostate carcinoma using ab32505 at 1/100 dilution.

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

Lane 1: 293T cell lysate transfected with GFP tagged AKT1
Lane 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

why is the actual band size different from the predicted?

Exposure time: 8 seconds

Blocking and diluting buffer and concentration: 5% NFDM/TBST
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 why is the actual band size different from the predicted?

Exposure time: 5 seconds

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

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 why is the actual band size different from the predicted?
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/1x10⁶ 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.

Please note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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