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
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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<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>
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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
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.

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
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
**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

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**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
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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