


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

**重组** RabMAb

★★★★★ **6 Abreviews** **139 References** **8 图像**

#### 概述

产品名称	Anti-AKT3 + AKT2 + AKT1抗体[Y89]
描述	兔单克隆抗体[Y89] to AKT3 + AKT2 + AKT1
宿主	Rabbit
特异性	This product reacts with AKT1, AKT2 and AKT3.
经测试应用	适用于: Flow Cyt (Intra), ICC/IF, WB, IHC-P, IP
种属反应性	与反应: Human 预测可用于: Mouse, Rat, Cow 
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	MCF7 cell lysate and prostate carcinoma tissue. IP: MCF7 cell lysate
常规说明	This product is a recombinant monoclonal antibody, which offers several advantages including: <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> For more information <a href="#">see here</a> . Our RabMAb <sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a> .

#### 性能

形式	Liquid
存放说明	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	Y89
同种型	IgG

## 应用

**The Abpromise guarantee**      **Abpromise™**承诺保证使用ab32505于以下的经测试应用

“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

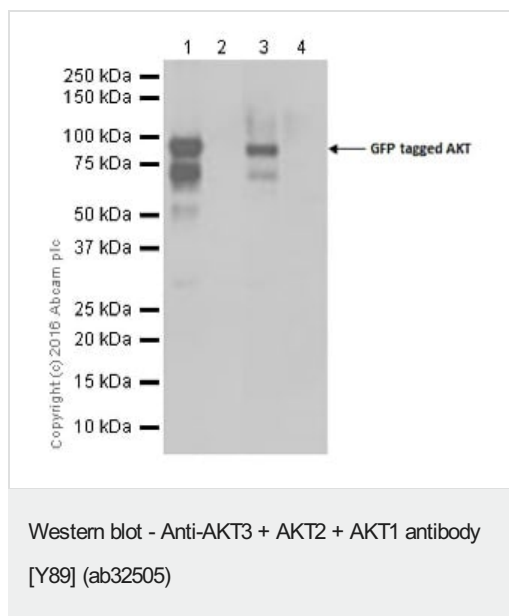
应用	Ab评论	说明
Flow Cyt (Intra)		1/20. <b>ab172730</b> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/100 - 1/250.
WB	★★★★★ (3)	1/2000 - 1/10000. Detects a band of approximately 59 kDa (predicted molecular weight: 56 kDa).
IHC-P	★★★★★ (1)	1/100. Perform heat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

## 靶标

### 细胞定位

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.

## 图片



**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

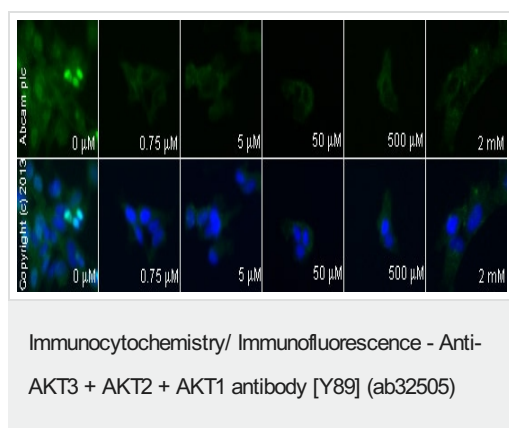
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

**Predicted band size:** 56 kDa

**Observed band size:** 82 kDa

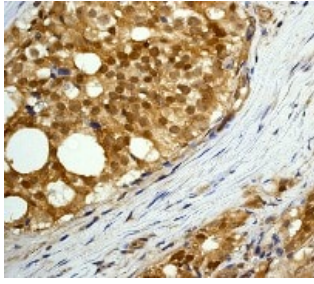
**Exposure time:** 8 seconds

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



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.

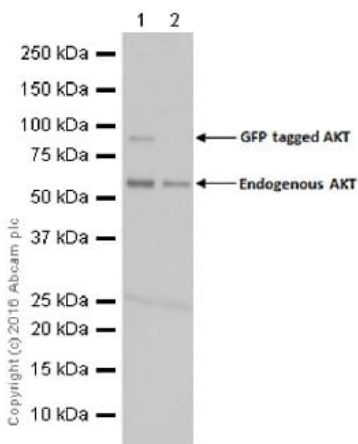
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32505 in MCF7 whole cell lysate.

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

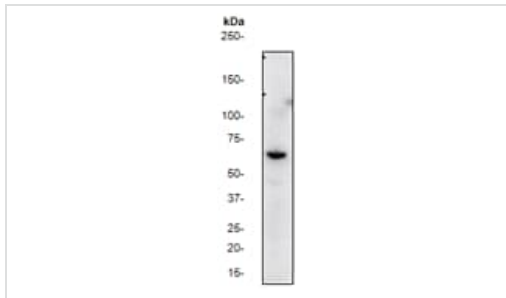
**All lanes** : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

**Predicted band size:** 56 kDa

**Observed band size:** 82 kDa

**Exposure time:** 5 seconds

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

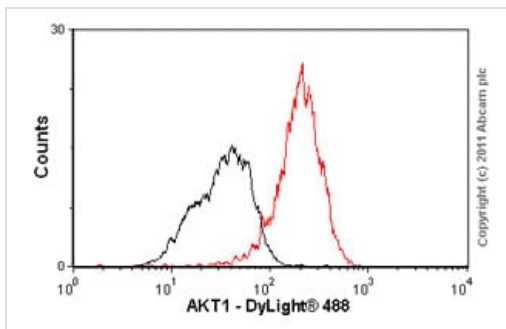


Western blot - Anti-AKT3 + AKT2 + AKT1 antibody [Y89] (ab32505)

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<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.

#### Why choose a recombinant antibody?



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

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