

# AKT [pS473] ABfinity™ Recombinant Rabbit Monoclonal Antibody - Purified



**REF** Catalog no. 700392

(See product label for lot information)

**Clone/PAD:** 98H9L8  
**Isotype:** IgG  
**Gene ID:** 207  
**Protein Acc. no.:** P31749  
**Qty:** 100 µg  
**Volume:** 200 µl  
**Concentration:** 0.5 mg/ml

## Formulation

PBS + 0.09% azide

## Immunogen

A peptide corresponding to amino acids 468-477 of P31749.

## Immunogen sequence

HFPQF[pS]YSAS

## Reactivity

This antibody reacts with human and mouse AKT1 [pS473]. Based on sequence identity and similarity, reactivity to rat and numerous other species is expected.

## Specificity

This antibody is specific for AKT1 [pS473] and does not recognize non-phosphorylated AKT

## Storage

2-8°C for up to 1 mo, -20°C for long term storage. Avoid repeated freezing and thawing.



## Expiration Date

Expires one year from date of receipt when stored as instructed.

## Validated Applications:

	Species	Test Material	Concentration
<b>Western Blotting</b>	mouse	NIH3T3 + PDGF	0.5-2 µg/ml
<b>Immunohistochemistry</b>	human	esophagus carcinoma	0.5-2 µg/ml
<b>Immunofluorescence</b>	mouse	NIH3T3 + insulin	4-6 µg/ml
<b>Flow Cytometry</b>	human	Jurkat + LY294002	1-2 µg/test
<b>Sandwich ELISA</b>	Detector		1-5 µg/ml

## Background

Akt, also known as protein kinase B (PKB) or RAC-alpha, is an ubiquitous serine/threonine kinase that plays an important role in diverse biological responses such as regulation of metabolism, cell survival and growth by phosphorylating multiple proteins (1). Akt is phosphorylated on threonine 308 by PDK1 and on serine 473 by PDK2. In addition to the two sites, phosphorylation at tyrosine 474 is required for full activation of Akt. The sequence used to make this antibody is conserved between Akt1, Akt2 and Akt3 in humans, as well as multiple other species.

## References

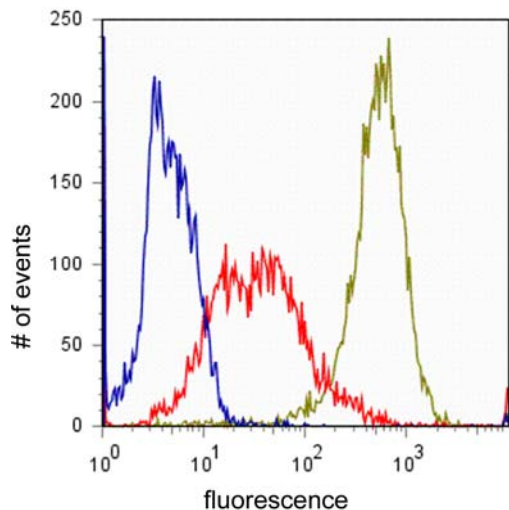
1. Sale, EM and Sale, GJ (2008) Review. Protein kinase B: signaling roles and therapeutic targeting. Cell Mol Life Sci 65:113-127.

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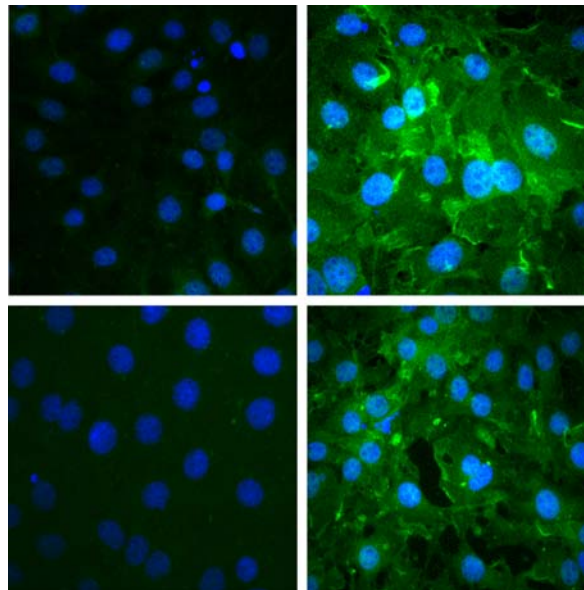
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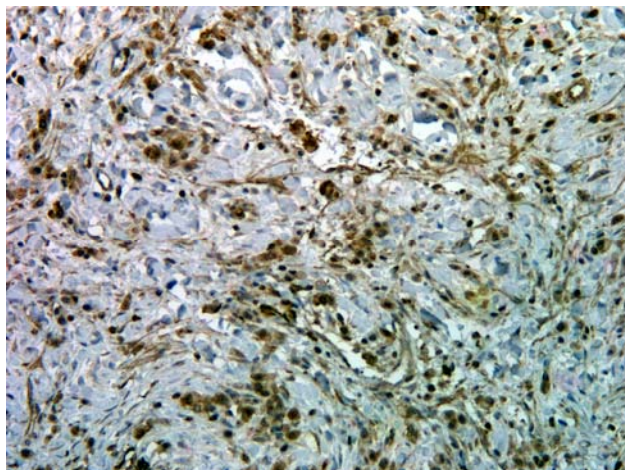
**Flow cytometry of Jurkat cells labeled with rabbit anti-AKT [pS473] (Cat. No. 700392).**

Jurkat cells were incubated with 50  $\mu$ M LY294002 (red trace) or without (green trace) for 1 h prior to being fixed and permeabilized using FIX & PERM® reagents (Cat. No. GAS004). Cells were then stained with 1  $\mu$ g/test anti-AKT [pS473] followed by Alexa Fluor® 488 goat anti-rabbit Ig [Cat. No. A11008]. The blue trace represents secondary antibody alone.



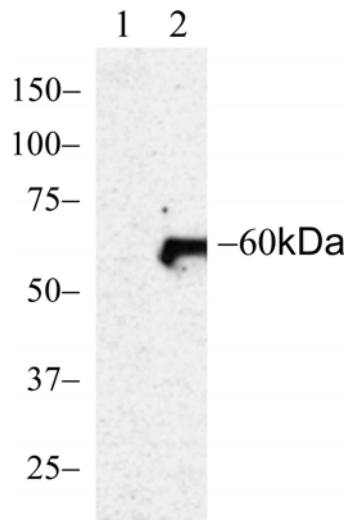
**Immunocytochemistry of mouse fibroblasts cells labeled with rabbit anti-AKT [pS473] (Cat. No. 700392).**

Mouse fibroblast cells were treated with (top right) or without (top left) 10  $\mu$ g/ml insulin and labeled with rabbit anti-AKT [pS473] (5  $\mu$ g/ml). Signal is knocked down after incubation with the phosphopeptide used as an immunogen (bottom left) but not with the non-phosphopeptide (bottom right). Alexa Fluor® 488 goat anti-rabbit (Cat. No. A11008) at 1:1000 was used as secondary antibody, Nuclei are stained with Hoechst (blue).



**Immunohistochemistry of human esophagus carcinoma tissue labeled with rabbit anti-AKT [pS473] (Cat. No. 700392).**

PE human esophagus carcinoma tissue was labeled with rabbit anti-AKT [pS473] (0.5  $\mu$ g/ml). Tissues were pretreated with EDTA and detected with SuperPicTure™ Polymer DAB (Cat. No.87-8963). Images were taken at 20x magnification. Note nuclear and cytoplasmic staining in tumor cells.



**Western blot of 3T3 lysates labeled with rabbit anti-AKT [pS473] (Cat. No. 700392).**

Rabbit anti-AKT [pS473] (0.1  $\mu$ g/ml) was used to label AKT [pS473] in untreated 3T3 lysates (lane 1) or PDGF treated 3T3 lysates (lane 2).

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