

Anti-phospho-Akt1/PKB α (Ser473), clone 11E6

Monoclonal Antibody

Cat. # 05-669

Lot # DAM1493402

pack size: 100 μ g

Store at -20°C

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS



Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, ELISA	H, M, R, Ca	IgG1k	N/A	M	60 kDa	NP_001014431

Background

The serine/threonine kinase Akt family contains several members, including Akt1 (also designated PKB or RacPK), Akt2 (also designated PKB- β or RacPK- β) and Akt3 (also designated PKB- γ or thymoma viral proto-oncogene 3), which exhibit sequence homology with the protein kinase A and C families and are encoded by the c-Akt proto-oncogene. All members of the Akt family have a Pleckstrin homology domain. Akt1 and Akt2 are activated by PDGF stimulation. This activation is dependent on PDGFR- β tyrosine residues 740 and 751, which bind the 85 kDa subunit of the phosphatidylinositol 3-kinase (PI 3-kinase) complex. Activation of Akt1 by insulin or insulin-growth factor-1(IGF-1) results in phosphorylation of both Thr 308 and Ser 473. Phosphorylation of both residues is important to generate a high level of Akt1 activity, and the phosphorylation of Thr 308 is not dependent on phosphorylation of Ser 473 in vivo. Thus, Akt proteins become phosphorylated and activated in insulin/IGF-1-stimulated cells by an upstream kinase(s). The activation of Akt1 and Akt2 is inhibited by the PI kinase inhibitor wortmannin. Taken together, this data strongly suggests that the protein signals downstream of the PI kinases.

Presentation

Purified mouse monoclonal IgG1k in buffer containing lyophilized serum-free cell culture supernatant by subsequent thiophilic adsorption and size exclusion chromatography from 1 mL storage buffer 2X PBS, PEG, sucrose, 0.09% sodium azide.

Concentration

1 mg/mL

Specificity

Recognizes phosphorylated Akt1/PKB α at Ser473, Mr 60 kDa. This lot of antibody does not cross-react with Akt1/PKB α dephosphorylated at Ser473.

Species Cross-reactivity

Human, mouse, canine and rat.

Immunogen

KLH conjugated synthetic peptide containing a pSer that corresponds to amino acid residues around phosphoserine 473.

Molecular Weight

60 kDa

Method of Purification

Thiophilic adsorption and size exclusion chromatography.

Storage and Handling

Stable for 1 year at -20°C from date of receipt.

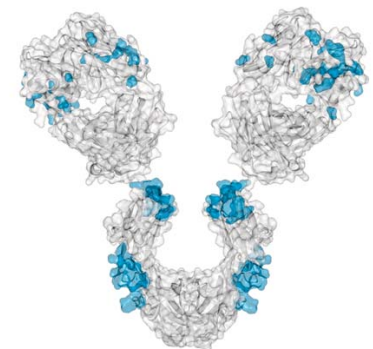
Handling Recommendations: Upon first thaw, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.

Control

PDGF treated NIH-3T3 cell lysates.

Quality Control Testing

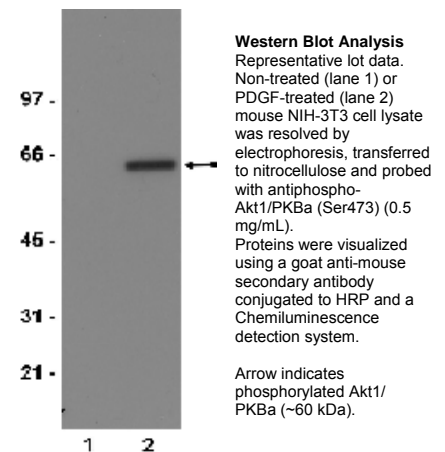
Routinely evaluated by western blot on lysates from mouse NIH-3T3 fibroblasts treated with PDGF.



References

1. Alessi, D.R., *et al.* (1998). *Curr Biol.* 8:69-81.
2. Cohen, P., *et al.* (1997). *FEBS Lett.* 410:3-10.
3. Alessi, D.R., *et al.* (1997). *Curr Biol.* 7:776-89.
4. James, S.R., *et al.* (1996). *Biochem J.* 315 (Pt 3):709-13.
5. Cross, D.A., *et al.* (1995). *Nature.* 378:785-9.

Western Blot Analysis: 0.5-2 μ g/mL of this lot detected phosphorylated Akt1/PKB α in lysates from mouse NIH-3T3 fibroblasts treated with 100 ng/mL PDGF for 20 minutes.



APPLICATION LEGEND: WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence
IH Immunohistochemistry (Tissue) ELISA Enzyme-linked Immunosorbent Assay

SPECIES LEGEND: Ca Canine (Dog) H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

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Additional Research Applications

ELISA: A previous lot was recommended at 0.05 mg/mL by an independent laboratory for ELISA.

PROTOCOL**Western Blot**

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50 mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1 μ g/mL each aprotinin, leupeptin, pepstatin; 1 mM Na₃VO₄; 1 mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared TBS containing 5% nonfat dry milk (Catalog # 20-200) and 0.05% Tween@-20 (TBST-MLK) for 1 hour at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 μ g/mL of anti-phospho-Akt1/PKB α (Ser473)** diluted in freshly prepared TBST-MLK for 2 hours with agitation at room temperature.
4. Wash the nitrocellulose three times with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG, Catalog # 12-349, 1:5000 dilution was used) in TBST-MLK for 1.5 hours at room temperature with agitation.
6. Rinse the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
8. Wash the nitrocellulose three times with water.
9. Use detection method of choice (enhanced chemiluminescence was used).

RELATED PRODUCTS (specific)

cat #	description
16-293	■ Anti-Akt/PKB, PH Domain, clone SKB1, Alexa Fluor® 488 conjugate
16-294	■ Anti-Akt/PKB, PH Domain, clone SKB1, Alexa Fluor® 555 conjugate
AB3137	■ Anti-Akt1
AB3132	■ Anti-Akt1, phospho-specific (Ser473)
07-416	■ Anti-Akt1/PKB α
05-796	■ Anti-Akt1/PKB α , clone AW24
06-885	■ Anti-Akt1/PKB α , PH domain, polyclonal
07-789	■ Anti-phospho-AKT (Thr34)
07-310	■ Anti-phospho-Akt1/PKB α (Ser473)
14-453	■ Akt1/PKB α (Δ PH, S473D), active
14-245	■ Akt1/PKB α , PH Domain (aa 1-149)
14-279	■ Akt1/PKB α , unactive
14-341	■ Akt1/PKB α , (PHD deletion), active
14-276	■ Akt1/PKB α , active
17-253	■ Akt1/PKB α cDNA (activated) Expression Kit
17-252	■ Akt1/PKB α cDNA (dominant negative) Expression Kit
17-254	■ Akt1/PKB α cDNA Allelic Pack
17-188	■ Akt1/PKB α Immunoprecipitation-Kinase Assay Kit
32-021	■ Akt1/PKB α KinEASE™ FP Fluorescein Green Assay
21-151	■ Akt1/PKB α cDNA (activated) in pUSEamp
21-152	■ Akt1/PKB α cDNA (dominant negative) in pUSEamp
21-153	■ Akt1/PKB α cDNA (wt) in pUSEamp

RELATED PRODUCTS (non-specific)

cat #	description
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 μ m
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 μ m
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 μ m
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0100	■ Immobilon Western Chemilum HRP Substrate 100 mL
17-373	■ Spray & Glow™ ECL WB Detection System 1 ea
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G

■ antibodies ■ Multiplex products ■ biotools ■ cell culture ■ enzymes ■ kits ■ proteins/peptides ■ siRNA/cDNA products

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