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

Monoclonal Antibody

Cat. # 05-669

Lot # DAM1598781

pack size: 100 µg

Store at -20℃

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Certificate of Analysis

Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/ Region	Host Species	Molecular Weight	Accession #
WB, ELISA	H, M, R, Ca	IgG1κ	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 thyoma 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/PKBα at Ser473, Mr 60 kDa. This lot of antibody does not cross-react with Akt1/PKBα 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℃ from date of receipt.

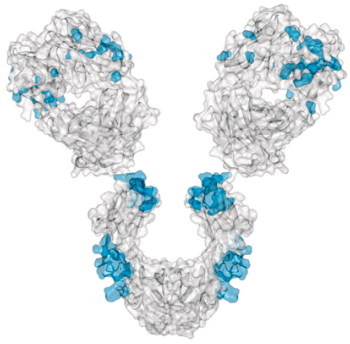
Handling Recommendations: Upon first thaw, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20℃. Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.

Control

PDGF treated NIH-3T3 cell lysates.

Quality Control Testing

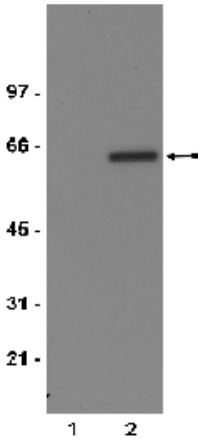
Routinely evaluated by western blot on lysates from mouse NIH-3T3 fibroblasts treated with PDGF.



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 µg/mL of this lot detected phosphorylated Akt1/PKBa in lysates from mouse NIH-3T3 fibroblasts treated with 100 ng/mL PDGF for 20 minutes.



Western Blot Analysis
Representative lot data. Non-treated (lane 1) or PDGF-treated (lane 2) mouse NIH-3T3 cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with antiphospho-Akt1/PKBa (Ser473) (0.5 mg/mL).
Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a Chemiluminescence detection system.

Arrow indicates phosphorylated Akt1/ PKBa (~60 kDa).

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

upstate | CHEMICON | Linco

THE EXPERTISE OF UPSTATE®, CHEMICON® AND LINCO® IS NOW A PART OF MILLIPORE

Please visit www.millipore.com for additional product information, test data and references.

Submit your published journal article, and earn credit toward future Millipore purchases. Visit www.millipore.com/publicationrewards to learn more!

Rev.A/2008-07-07/05-669CA/JM

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 µg/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 µg/mL of anti-phospho-Akt1/PKBa (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/PKBα
05-796	Anti-Akt1/PKBα, clone AW24
06-885	Anti-Akt1/PKBα, PH domain, polyclonal
07-789	Anti-phospho-AKT (Thr34)
07-310	Anti-phospho-Akt1/PKBα (Ser473)
14-453	Akt1/PKBα (ΔPH, S473D), active
14-245	Akt1/PKBα , PH Domain (aa 1-149)
14-279	Akt1/PKBα, unactive
14-341	Akt1/PKBα, (PHD deletion), active
14-276	Akt1/PKBα, active
17-253	Akt1/PKBα cDNA (activated) Expression Kit
17-252	Akt1/PKBα cDNA (dominant negative) Expression Kit
17-254	Akt1/PKBα cDNA Allelic Pack
17-188	Akt1/PKBα Immunoprecipitation-Kinase Assay Kit
32-021	Akt1/PKBα KinEASE™ FP Fluorescein Green Assay
21-151	Akt1/PKBα cDNA (activated) in pUSEamp
21-152	Akt1/PKBα cDNA (dominant negative) in pUSEamp
21-153	Akt1/PKBα cDNA (wt) in pUSEamp

RELATED PRODUCTS (non-specific)

cat #	description
IPVH00010	Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPFL00010	Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm
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 µm
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