

Anti-phospho-Akt (Thr308) (IHC only)



Polyclonal Antibody

Cat. # 07-1398

Lot # 2147123

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

pack size: 100 µL

Store at 2-8°C

Certificate of Analysis

page 1 of 2

Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
IH(P)	H, M (R, B, Ch)	N/A	Thr308 (Catalytic Domain)	Rb	60 kDa	NP_001014431

Background

Akt/PKB is a Ser/Thr kinase and a major known effector of the PI3 Kinase pathway. In mammals, three isoforms of Akt (Akt1/PKC α , Akt2/PKB β , and Akt3/PKB γ) exist. They exhibit a high degree of homology, but differ slightly in the localization of their regulatory phosphorylation sites. Akt1 is the predominant isoform that is in most tissues and is thought to have a dominant role in growth, survival, embryonic development, and adipocyte differentiation. Akt2 is correlated with the regulation of glucose homeostasis and is the predominant isoform expressed in insulin-responsive tissues. Akt3 is abundant in brain tissue. Each Akt isoform is composed of three functionally distinct regions: an N-terminal Pleckstrin Homology (PH) domain that provides a lipid-binding module, a central catalytic domain containing Thr308, and a C-terminal hydrophobic motif containing Ser473. The activation of AKT is dependent on a dual regulatory mechanism that requires both its translocation to the plasma membrane and dual phosphorylation on Thr308 and Ser473 by PDK1 and the TORC2 complex, respectively.

Presentation

Antigen affinity purified rabbit serum in 0.1M Tris-Glycine (pH7.4), 150mM NaCl, with 0.05% NaN₃.

Concentration

0.5 mg/mL

Specificity

Detects Akt only when phosphorylated on Thr308.

Species Cross-reactivity

Human and Mouse tested. Expected to react with Rat, Chicken, Dog, Xenopus, and Cow based on 100% immunogen sequence

homology.

Immunogen

KLH-conjugated synthetic peptide containing the Thr308 sequence and surrounding amino acids of human, mouse, rat, bovine and chicken Akt1. Human and mouse Akt2 shares 11/12 amino acids and rat Akt3 shares 10/12 amino acids with the peptide immunogen sequence.

Molecular Weight

60 kDa

Method of Purification

Antigen affinity purified against both the phosphorylated and the non-phosphorylated peptides of Akt (Thr308).

Storage and Handling

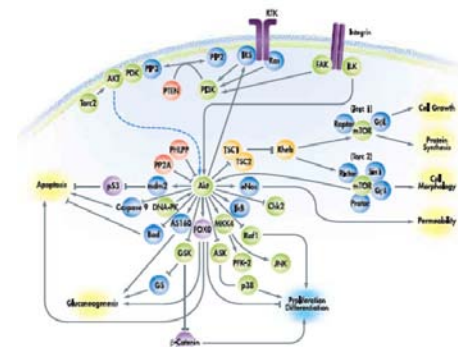
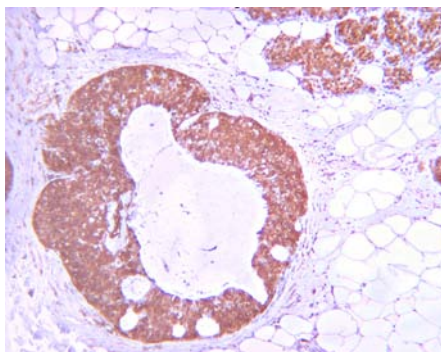
Stable for 1 year at 2-8°C from date of receipt.

Handling Recommendations: Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution.

Quality Control Testing

Immunohistochemistry Analysis:

This antibody detected Akt when phosphorylated on Thr308 at a 1:500 dilution in paraffin-embedded human ductal breast carcinoma.



References

General References:

- Alessi D.R., *et al.*, EMBO 15: 6541-6551, 1996.
- Cross, D.A., *et al.*, Nature 378: 785-789, 1995.
- James, S.R., *et al.*, Biochem. J. 315: 709-713, 1996.
- Burgering and Coffer, Nature 376: 599-601, 1995.
- Ackler, S., *et al.*, Oncogene 21: 198-206, 2002.

Immunohistochemistry Analysis:

Representative lot data.

Formalin-fixed and paraffin-embedded Human ductal breast carcinoma with citrate pH 6.0 (HIER) stained with Anti-phospho-Akt (Thr308) a dilution of 1:500. Tissue pretreated with citrate pH 6, antigen retrieval. Antibody diluted to 1:500, IHC-Select Detection with HRP-DAB.

APPLICATION LEGEND: WB Western Blotting IP Immunoprecipitation IF Immunofluorescence FC Flow Cytometry

IH(P) Immunohistochemistry (Paraffin) ELISA Enzyme-Linked Immunosorbent Assay

SPECIES LEGEND: H Human M Mouse R Rat Rb Rabbit B Bovine Ch Chicken WR Most Common Vertebrates

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/publication_rewards to learn more!

upstate | CHEMICON | Linco

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

PROTOCOL**Immunohistochemistry**

1. Use standard de-paraffinization techniques on tissue specimens.
2. Pre-treat tissues using a citrate buffer, pH 6.0 and high heat epitope retrieval techniques.

Note: Do not allow tissues to dry out during the staining procedure.

The following steps are taken from the product manual for IHC Select® HRP/DAB Detection Kit (Cat. No. DAB050)

3. Apply the blocking reagent to the tissue specimen and incubate in an enclosed chamber for 5 minutes.
4. While holding the slide at a 45° angle, gently rinse the specimen with 1X Rinse Buffer for a minimum of 15 seconds. Tap the end of the slide onto a paper towel to remove excess Rinse Buffer.
5. Apply a 1:50-1:500 dilution of primary antibody over the entire tissue specimen and incubate in an enclosed chamber at room temperature for 60 minutes.
6. Rinse specimen as performed in Step 4.
7. Apply the biotinylated secondary antibody to the tissue specimen and incubate in an enclosed chamber for 10 minutes.
8. Rinse specimen as performed in Step 4.
9. Apply the Streptavidin-HRP solution to the tissue specimen and incubate in an enclosed chamber for 10 minutes.
10. Rinse specimen as performed in Step 4.
11. Apply the DAB (chromogen reagent) to the tissue specimen and incubate in an enclosed chamber for 10 minutes.
12. Rinse specimen as performed in Step 4.
13. Apply the Hematoxylin counterstain solution to the tissue specimen and incubate in an enclosed chamber for 1 minute.
14. Rinse specimen as performed in Step 4.
15. Place the tissue slides directly into a container filled with deionized water until mounting.
16. Mount a coverslip using an aqueous-based mounting media or for permanent mounting, dehydrate tissue through a graded series of alcohols, immerse in xylene, then apply a xylene-based mounting media (e.g. Permount) and coverslip.

RELATED PRODUCTS (specific)

cat #	description
05-802R	■ Anti-phospho-Akt (Thr308), recombinant Rabbit Monoclonal
05-591	■ Anti-Akt/PKB, PH Domain, clone SKB1
15-101	■ Akt Pathway Explorer Sample Pack
15-103	■ PI3K/Akt/GSK Pathway Explorer Sample Pack
15-104	■ Akt/mTOR/S6K Pathway Explorer Sample Pack
16-185	■ Anti-Akt/PKB, PH Domain, agarose conjugate
05-591MG	■ Anti-Akt/PKB, PH Domain, clone SKB1
17-444	■ Akt (Ser473) Dual Detect CELISA Assay Kit (Fluorogenic Detection)
17-455	■ STAR AKT1 ELISA Kit
17-457	■ STAR phospho-Akt (Ser473) ELISA Kit
17-456	■ STAR phospho-Akt (Thr308) ELISA Kit
16-293	■ Anti-Akt/PKB, PH Domain, clone SKB1, Alexa Fluor® 488 conjugate
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)
16-305	■ Anti-phospho-Akt1/PKBα (Ser473), clone 11E6, Alexa Fluor® 488 conjugate
16-306	■ Anti-phospho-Akt1/PKBα (Ser473), clone 11E6, Alexa Fluor® 555 conjugate
05-736	■ Anti-phospho-Akt1/PKBα (Ser473), clone SK703
06-678	■ Anti-phospho-Akt1/PKBα (Thr308)
05-669	■ Anti-phospho-Akt1/PKBα (Ser473), clone 11E6
14-245	■ Akt1/PKBα, PH Domain (aa 1-149)
14-276	■ Akt1/PKBα, active
14-279	■ Akt1/PKBα, inactive
17-188	■ Akt1/PKBα Immunoprecipitation-Kinase Assay Kit
05-771	■ Anti-Akt2/PKBβ, clone AW114
05-780	■ Anti-Akt3/PKBγ, clone GMA104
05-988	■ http://www.millipore.com/catalogue/item/05-988 Anti-PRAS40
07-888	■ Anti-phospho-PRAS40 (Thr246)

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

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

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

28820 Single Oak Drive • Temecula, CA 92590

Technical Support: T: 1-800-MILLIPORE (1-800-645-5476) • F: 1-800-437-7502

FOR RESEARCH USE ONLY. Not for use in diagnostic or therapeutic applications. Purchase of this Product does not include any right to resell or transfer, either as a stand-alone product or as a component of another product. Any use of this Product for purposes other than research is strictly prohibited without prior written authorization from an authorized officer of Millipore Corporation.

Upstate®, Chemicon®, Linco® and all other trademarks are owned by Millipore Corporation. Copyright ©2008 Millipore Corporation. All rights reserved.



We Buy 100% Certified Renewable Energy