


#7144 Store at 4°C

PathScan® Phospho-Akt (Thr308) Sandwich ELISA Antibody Pair



1 Kit (4 x 96 assays)

Species Cross Reactivity
H M

UniProt ID:
#P31751, #Q9Y243,
#P31749

Entrez-Gene Id:
#208, #10000,
#207

Orders: 877-616-CELL (2355)
orders@cellsignal.com

Support: 877-678-TECH (8324)

Web: info@cellsignal.com
cellsignal.com

3 Trask Lane | Danvers | Massachusetts | 01923 | USA

For Research Use Only. Not for Use in Diagnostic Procedures.

Product Includes	Product #	Volume	Cap Color	Storage Temp
Akt Capture Rabbit mAb (100X)	30257	400 µl	Pink	4°C
Phospho-Akt (Thr308) Detection Mouse mAb (100X)	96108	400 µl	Blue	4°C
Anti-mouse IgG, HRP-linked Antibody (1000X)	20725	40 µl	Yellow	-20°C

Please visit cellsignal.com for a complete listing of recommended companion products.

Description

CST's PathScan® Phospho-Akt (Thr308) Sandwich ELISA Antibody Pair is offered as an economical alternative to our PathScan® Phospho-Akt (Thr308) Sandwich ELISA Kit #7252. Capture and Detection Antibodies (100X stocks) and HRP-Conjugated Secondary Antibody (1000X stock) are supplied. Sufficient reagents are supplied for 4 x 96 well ELISAs. The Akt Rabbit Capture Antibody is coated in PBS overnight in a 96 well microplate. After blocking, cell lysates are added, followed by Phospho-Akt (Thr308) Mouse Detection Antibody and HRP-conjugated Anti-Mouse IgG. HRP substrate, TMB, is added for color development. The magnitude of the absorbance for this developed color is proportional to the quantity of phospho-Akt (Thr308) protein.

*Antibodies in kit are custom formulations specific to kit.

Reagents not supplied

Phosphate Buffered Saline (PBS-20X) #9808
 Phosphate Buffered Saline with Tween-20 (PBST-20X) #9809
 Cell Lysis Buffer (10X) #9803
 TMB Substrate #7004
 STOP Solution #7002

Blocking Buffer: 1X PBS/0.5% Tween-20, 1% BSA

96 Well Microplates**

Microplate Reader

** Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592).

Notes: Antibody pairs have been optimized using recommended buffers, reagents, plates and the included protocol. Solutions should be made fresh daily.

Background

Akt, also referred to as PKB or Rac, plays a critical role in controlling cell survival and apoptosis (1-3). This protein kinase is activated by insulin and various growth and survival factors to function in a wortmannin-sensitive pathway involving PI3 kinase (2,3). Akt is activated by phospholipid binding and activation loop phosphorylation at Thr308 by PDK1 (4) and by phosphorylation within the carboxy terminus at Ser473. The previously elusive PDK2 responsible for phosphorylation of Akt at Ser473 has been identified as mammalian target of rapamycin (mTOR) in a rapamycin-insensitive complex with rictor and Sin1 (5,6). Akt promotes cell survival by inhibiting apoptosis through phosphorylation and inactivation of several targets, including Bad (7), forkhead transcription factors (8), c-Raf (9), and caspase-9. PTEN phosphatase is a major negative regulator of the PI3K/Akt signaling pathway (10). LY294002 is a specific PI3 kinase inhibitor (11). Another essential Akt function is the regulation of glycogen synthesis through phosphorylation and inactivation of GSK-3α and β (12,13). Akt may also play a role in insulin stimulation of glucose transport (12). In addition to its role in survival and glycogen synthesis, Akt is involved in cell cycle regulation by preventing GSK-3β-mediated phosphorylation and degradation of cyclin D1 (14) and by negatively regulating the cyclin-dependent kinase inhibitors p27 Kip1 (15) and p21 Waf1/Cip1 (16). Akt also plays a critical role in cell growth by directly phosphorylating mTOR in a rapamycin-sensitive complex containing raptor (17). More importantly, Akt phosphorylates and inactivates tuberlin (TSC2), an inhibitor of mTOR within the mTOR-raptor complex (18,19).

Background References

1. Franke, T.F. et al. (1997) *Cell* 88, 435-7.
2. Burgering, B.M. and Coffey, P.J. (1995) *Nature* 376, 599-602.
3. Franke, T.F. et al. (1995) *Cell* 81, 727-36.
4. Alessi, D.R. et al. (1996) *EMBO J* 15, 6541-51.
5. Sarbassov, D.D. et al. (2005) *Science* 307, 1098-101.

6. Jacinto, E. et al. (2006) *Cell* 127, 125-37.
7. Cardone, M.H. et al. (1998) *Science* 282, 1318-21.
8. Brunet, A. et al. (1999) *Cell* 96, 857-68.
9. Zimmermann, S. and Moelling, K. (1999) *Science* 286, 1741-4.
10. Cantley, L.C. and Neel, B.G. (1999) *Proc Natl Acad Sci USA* 96, 4240-5.
11. Vlahos, C.J. et al. (1994) *J Biol Chem* 269, 5241-8.
12. Hajdich, E. et al. (2001) *FEBS Lett* 492, 199-203.
13. Cross, D.A. et al. (1995) *Nature* 378, 785-9.
14. Diehl, J.A. et al. (1998) *Genes Dev* 12, 3499-511.
15. Gesbert, F. et al. (2000) *J Biol Chem* 275, 39223-30.
16. Zhou, B.P. et al. (2001) *Nat Cell Biol* 3, 245-52.
17. Navé, B.T. et al. (1999) *Biochem J* 344 Pt 2, 427-31.
18. Inoki, K. et al. (2002) *Nat Cell Biol* 4, 648-57.
19. Manning, B.D. et al. (2002) *Mol Cell* 10, 151-62.

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken **Dm:** D. melanogaster
X: Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** horse
GP: Guinea Pig **Rab:** rabbit **All:** all species expected

Trademarks and Patents

Cell Signaling Technology is a trademark of Cell Signaling Technology, Inc.
 PathScan is a registered trademark of Cell Signaling Technology, Inc.
 U.S. Patent No. 7,429,487, foreign equivalents, and child patents deriving therefrom.
 All other trademarks are the property of their respective owners. Visit cellsignal.com/trademarks for more information.

Limited Uses

Except as otherwise expressly agreed in a writing signed by a legally authorized representative of CST, the following terms apply to Products provided by CST, its affiliates or its distributors. Any Customer's terms and conditions that are in addition to, or different from, those contained herein, unless separately accepted in writing by a legally authorized representative of CST, are rejected and are of no force or effect.

Products are labeled with For Research Use Only or a similar labeling statement and have not been approved, cleared, or licensed by the FDA or other regulatory foreign or domestic entity, for any purpose. Customer shall not use any Product for any diagnostic or therapeutic purpose, or otherwise in any manner that conflicts with its labeling statement. Products sold or licensed by CST are provided for Customer as the end-user and solely for research and development uses. Any use of Product for diagnostic, prophylactic or therapeutic purposes, or any purchase of Product for resale (alone or as a component) or other commercial purpose, requires a separate license from CST. Customer shall (a) not sell, license, loan, donate or otherwise transfer or make available any Product to any third party, whether alone or in combination with other materials, or use the Products to manufacture any commercial products, (b) not copy, modify, reverse engineer, decompile, disassemble or otherwise attempt to discover the underlying structure or technology of the Products, or use the Products for the purpose of developing any products or services that would compete with CST products or services, (c) not alter or remove from the Products any trademarks, trade names, logos, patent or copyright notices or markings, (d) use the Products solely in accordance with CST Product Terms of Sale and any applicable documentation, and (e) comply with any license, terms of service or similar agreement with respect to any third party products or services used by Customer in connection with the Products.

#7144

PathScan® Phospho-Akt (Thr308) Sandwich ELISA Antibody Pair



ELISA Antibody Pair

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH₂O, mix.
2. **Wash Buffer:** 1X PBS/0.05% Tween® 20, (20X PBST #9809).
3. **Blocking Buffer:** 1X PBS/0.05% Tween® 20, 1% BSA.
4. **1X Cell Lysis Buffer:** 10X Cell Lysis Buffer (#9803): To prepare 10 ml of 1X Cell Lysis Buffer, add 1 ml of 10X Cell Lysis Buffer to 9 ml of dH₂O, mix. Buffer can be stored at 4°C for short-term use (1–2 weeks).

Recommended: Add 1 mM phenylmethylsulfonyl fluoride (PMSF) (#8553) immediately before use.

5. **Bovine Serum Albumin (BSA):** (#9998).
6. **TMB Substrate:** (#7004).
7. **STOP Solution:** (#7002)

NOTE: Reagents should be made fresh daily.

B. Preparing Cell Lysates

For adherent cells

1. Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM PMSF to each plate (10 cm diameter) and incubate the plate on ice for 5 min.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 min (x14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at -80°C in single-use aliquots.

For suspension cells

1. Remove media by low speed centrifugation (~1,200 rpm) when the culture reaches 0.5–1.0 x 10⁶ viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1,200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
3. Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X cell lysis buffer plus 1 mM PMSF.
4. Sonicate lysates on ice.
5. Microcentrifuge for 10 min (x14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at -80°C in single-use aliquots.

C. Coating Procedure

1. Rinse microplate with 200 µl of dH₂O, discard liquid. Blot on paper towel to make sure wells are dry.
2. Dilute capture antibody 1:100 in 1X PBS. For a single 96 well plate, add 100 µl of capture antibody stock to 9.9 ml 1X PBS. Mix well and add 100 µl/well. Cover plate and incubate overnight at 4°C (17–20 hr).
3. **After overnight coating, gently uncover plate and wash wells:**
 1. Discard plate contents into a receptacle.
 2. Wash four times with wash buffer, 200 µl each time per well. For each wash, strike plates on fresh paper towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
 3. Clean the underside of all wells with a lint-free tissue.
4. Block plates. Add 150 µl of blocking buffer/well, cover plate, and incubate at 37°C for 2 hr.
5. After blocking, wash plate (Section C, Step 3). Plate is ready to use.

D. Test Procedure

1. Lysates can be used undiluted or diluted in blocking buffer. 100 µl of lysate is added per well. Cover plate and incubate at 37°C for 2 hr.
2. Wash plate (Section C, Step 3).
3. Dilute detection antibody 1:100 in blocking buffer. For a single 96 well plate, add 100 µl of detection antibody Stock to 9.9 ml of blocking buffer. Mix well and add 100 µl/well. Cover plate and incubate at 37°C for 1 hr.
4. Wash plate (Section C, Step 3).
5. Secondary antibody, either streptavidin anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in blocking buffer. For a single 96 well plate, add 10 µl of secondary antibody stock to 9.99 ml of blocking buffer. Mix well and add 100 µl/well. Cover and incubate at 37°C for 30

min.

6. Wash plate (Section C, Step 3).

7. Add 100 µl of TMB substrate per well. Cover and incubate at 37°C for 10 min.

8. Add 100 µl of STOP solution per well. Shake gently for a few seconds.

9. Read plate on a microplate reader at absorbance 450 nm.

1. **Visual Determination:** Read within 30 min after adding STOP solution.

2. **Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP solution.

posted January 2008

revised September 2013

Orders: 877-616-CELL (2355) • orders@cellsignal.com • Support: 877-678-TECH (8324) • info@cellsignal.com • Web: cellsignal.com
For Research Use Only. Not for Use in Diagnostic Procedures.