Store at 4°C

#9474

PathScan® Akt Signaling Antibody Array Kit (Chemiluminescent Readout)

Cell Signaling TECHNOLOGY®

☑ 1 Kit
(32 multiplexed assays)

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Support ■ 877-678-TECH (8324)
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rev. 04/14/16

For Research Use Only. Not For Use In Diagnostic Procedures.

Species Cross-Reactivity: H, M

Description: The PathScan® Akt Signaling Antibody Array Kit (Chemiluminescent Readout) uses glass slides as the planar surface and is based upon the sandwich immunoassay principle. The array kit allows for the simultaneous detection of 16 phosphorylated proteins predominantly belonging to the Akt signaling network. Target-specific capture antibodies have been spotted in duplicate onto nitrocellulose-coated glass slides. Each kit contains two 16-pad slides, allowing the user to test up to 32 samples and generate 512 data points in a single experiment. Cell lysate is incubated on the slide followed by a biotinylated detection antibody cocktail. Streptavidin-conjugated HRP and LumiGLO® Reagent are then used to visualize the bound detection antibody by chemiluminescence. An image of the slide can be captured with either a digital imaging system or standard chemiluminescent film. The image can be analyzed visually or the spot intensities quantified using array analysis software.

Specificity/Sensitivity: PathScan® Akt Signaling Antibody Array Kit (Chemiluminescent Readout) detects the indicated cellular proteins and signaling nodes only when phosphorylated at the specified residues (see Array Target Map). No substantial cross-reactivity has been observed between targets. This kit is optimized for cell lysates diluted to a total protein concentration between 0.2 and 1 mg/ml (see kit protocol).

Products Included	Quantity	Cap Color
Array Slides	2 slides	
Multi-Well Gasket	2 gaskets	
Sealing Tape	2 sheets	
Chemiluminescent Development Folder	2 folders	
20X Array Wash Buffer	15 ml	White
Array Blocking Buffer	5 ml	Red
Array Diluent Buffer	15 ml	Blue
10X Detection Antibody Cocktail	300 µl	White
10X HRP-linked Streptavidin	300 µl	Clear
20X LumiGLO® Reagent A #7003	5 ml	Brown
20X Peroxide Reagent B #7003	5 ml	Clear
*Cell Lysis Buffer #7018	30 ml	Clear

*Kit should be stored at 4°C with the exception of 1X Cell Lysis Buffer, which is stored at –20°C (packaged separately).

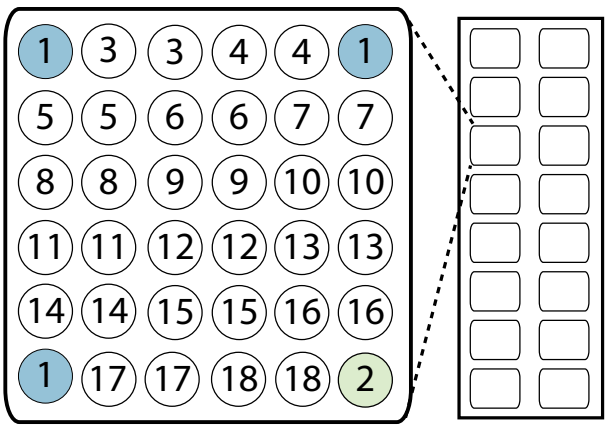


Figure 1. Target map of the PathScan® Akt Signaling Antibody Array Kit (Chemiluminescent Readout) #9474.

Akt Signaling

	Target	Phosphorylation Site
1	Positive Control	N/A
2	Negative Control	N/A
3	Akt	Thr308
4	Akt	Ser473
5	S6 Ribosomal Protein	Ser235/236
6	AMPKα	Thr172
7	PRAS40	Thr246
8	mTOR	Ser2481
9	GSK-3α	Ser21
10	GSK-3β	Ser9
11	p70 S6 Kinase	Thr389
12	p70 S6 Kinase	Thr421/Ser424
13	Bad	Ser112
14	RSK1	Thr421/Ser424
15	PTEN	Ser380
16	PDK 1	Ser241
17	Erk1/2	Thr202/Tyr204
18	4E-BP1	Thr37/46

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

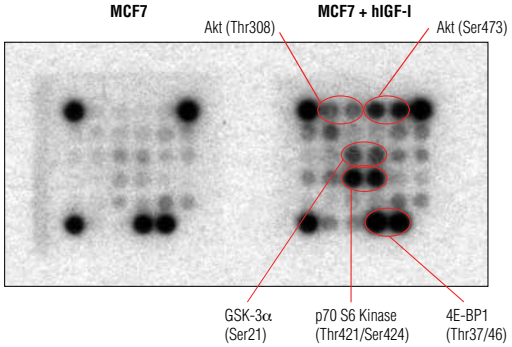


Figure 2. MCF7 cells were grown to 85% confluency and then serum starved overnight. Cells were either untreated (left panel) or treated with Human Insulin-like Growth Factor I (hIGF-I) #8917 (100 ng/ml, 20 min; right panel). Cell extracts were prepared and analyzed using the PathScan® Akt Signaling Antibody Array Kit (Chemiluminescent Readout) #9474. Images were acquired by briefly exposing the slide to standard chemiluminescent film.

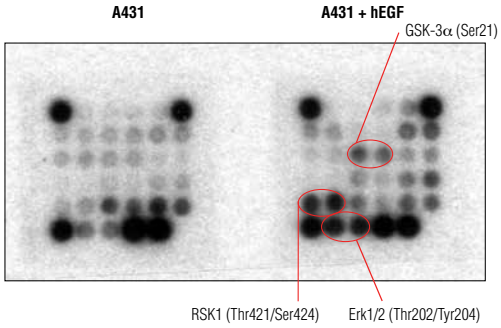


Figure 3. A431 cells were grown to 85% confluency and then serum starved overnight. Cells were either untreated (left panel) or treated with Human Epidermal Growth Factor (hEGF) #8916 (100 ng/ml, 5 min; right panel). Cell extracts were prepared and analyzed using the PathScan® Akt Signaling Antibody Array Kit (Chemiluminescent Readout) #9474. Images were acquired by briefly exposing the slide to standard chemiluminescent film.

Background: The Akt signaling module is typically activated in response to growth factor stimulation of receptor tyrosine kinases transmitting primarily anabolic growth and survival signals. Akt1/2 are ubiquitously expressed protein kinases having a multitude of cellular substrates and are involved in the regulation of a wide range of cellular processes. Akt is activated by phosphorylation at two distinct sites: Ser473 by the mTORC2 complex and Thr308 by the plasma membrane residing kinase PDK1.

PI3 kinase is a lipid kinase that phosphorylates inositol phospholipids at position three to generate docking sites for Akt at the plasma membrane where Akt is activated. PTEN is a lipid phosphatase that negates the action of PI3 kinase to downregulate the signal emanating from this module.

mTOR integrates growth factor signaling and nutrient availability and is a core component of two macromolecular complexes, mTORC1 and mTORC2. The autophosphorylation of mTOR at Ser2481 correlates with the levels of its activation. mTORC1 phosphorylation of p70 S6 kinase leads to kinase activation, which in turn activates protein synthesis. The S6 ribosomal protein is found downstream of p70 S6 kinase and its phoshporylation at Ser235/236 reflects mTOR pathway activation. The mTORC2 complex activates Akt by phosphorylating it at Ser473. Phosphorylation of PRAS40 at Thr246 by Akt relieves PRAS40 inhibition of mTORC1.

4E-BP1 is a repressor of translation and inhibits cap-dependent translation initiation. Hyperphosphorylation of 4E-BP1 by mTORC1 leads to derepression of this blockade, which results in activation of cap-dependent translation.

Phosphorylation of the pro-apoptotic protein Bad at Ser112 and the multifunctional kinases GSK-3α and GSK-3β at Ser21 and Ser9, respectively, by Akt inhibits their activity and promotes cell survival.

AMPK is an energy sensor that is activated by phosphorylation at Thr172 in response to elevated AMP levels. Under conditions of low energy and elevated levels of AMP, AMPK helps to ensure that anabolic processes, such as those triggered by Akt, are decreased until energy levels are restored.

Although not a component of the Akt signaling network, Erk1 and Erk2 kinases are a central component of the Ras/MAP kinase signaling module. Erk1/2 regulate multiple cellular functions and are involved in a broad range of cellular processes, such as proliferation, differentiation, and motility. Erk and Akt signaling modules cross regulate each other at multiple points and through a variety of mechanisms. Erk is activated by a wide range of extracellular signals including growth factors, cytokines, hormones, and neurotransmitters, leading to dual phosphorylation at Thr202 and Tyr204.

The 90 kDa ribosomal S6 kinase 1 (RSK1) is activated primarily by Erk1/2 in response to many growth factors, polypeptide hormones, and neurotransmitters. p90RSK1 phosphorylates a wide range of substrates including ribosomal protein S6, and positively regulates protein translation and cellular growth. p90RSK1 can also be activated by kinases that regulate the response to cellular stress.

Background References:

(1) Lawlor, M.A. and Alessi, D.R. (2001) *J Cell Sci* 114, 2903-10.

(2) Brazil, D.P. and Hemmings, B.A. (2001) *Trends Biochem Sci* 26, 657-64.

(3) Brazil, D.P. et al. (2002) *Cell* 111, 293-303.

(4) Luo, J. et al. (2003) *Cancer Cell* 4, 257-62.

(5) Manning, B.D. and Cantley, L.C. (2007) *Cell* 129, 1261-74.

(6) Franke, T.F. (2008) *Sci Signal* 1, pe29.

(7) Huang, J. and Manning, B.D. (2009) *Biochem Soc Trans* 37, 217-22.

(8) Hers, I. et al. (2011) *Cell Signal* 23, 1515-27.

(9) Dufner, A. and Thomas, G. (1999) *Exp Cell Res* 253, 100-9.

(10) Rubinfeld, H. and Seger, R. (2005) *Mol Biotechnol* 31, 151-74.

(11) Mihaylova, M.M. and Shaw, R.J. (2011) *Nat Cell Biol* 13, 1016-23.

(12) Hardie, D.G. et al. (2012) *Nat Rev Mol Cell Biol* 13, 251-62.

PathScan® Akt Signaling Antibody Array Kit (Chemiluminescent Readout) Protocol

A Preparing Cell Lysates

1. Thaw 1X Cell Lysis Buffer #7018 and mix thoroughly. Supplement Cell Lysis Buffer with phenylmethylsulfonyl fluoride (PMSF) to a final concentration of 1 mM, or a cocktail of protease inhibitors (not included). Keep lysis buffer on ice.
2. Remove media and wash cells once with ice-cold 1X PBS.
3. Remove PBS and add ice-cold Cell Lysis Buffer. For adherent cells, use 0.5 ml cell lysis buffer for each plate (10 cm in diameter). Incubate on ice for 2 minutes.
4. Tilt the plate and collect the lysate into a clean micro tube.
5. Optional step: microcentrifuge the lysate at maximum speed for 3 minutes at 4°C and transfer the supernatant to a new tube. This step is usually not required but can help remove any particles or large cell debris, if present. Lysate may be used immediately or stored at - 80°C in single-use aliquots.
6. Immediately before performing the assay, dilute lysates to 0.2 – 1.0 mg/ml in Array Diluent Buffer. Set aside on ice.

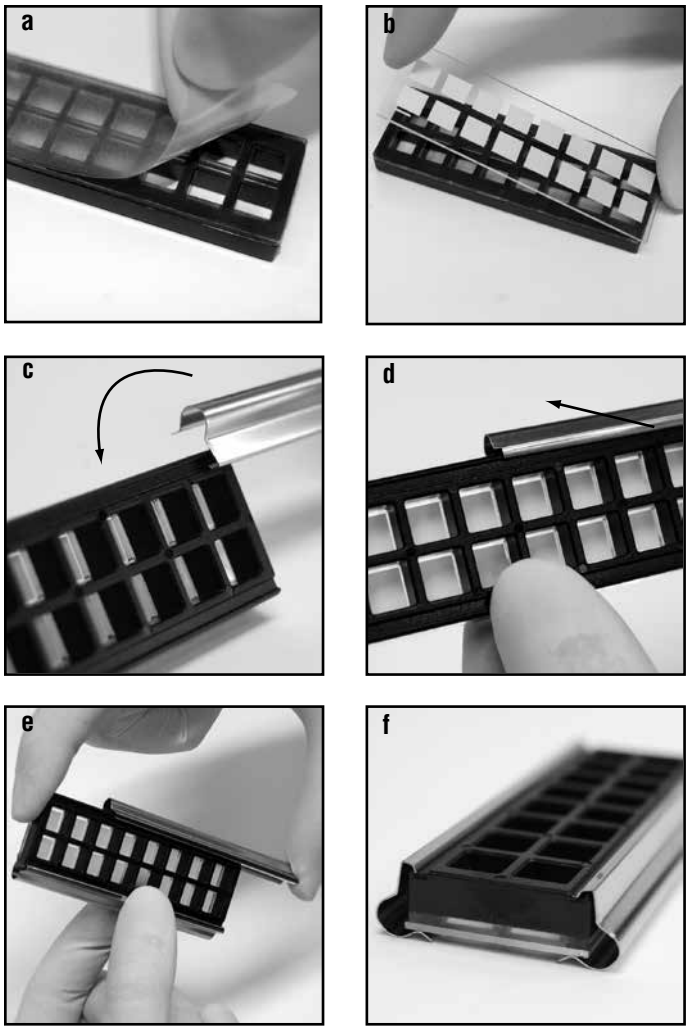
B Assay Procedure

1. Bring glass slides and blocking buffer to room temperature before use.
2. Prepare 1X Array Wash Buffer by diluting 20X Array Wash Buffer in deionized water. Keep at room temperature. Dilute 1 mL of 20X Array Wash Buffer with 19 mL of deionized water. Label as 1X Array Wash Buffer.
3. Prepare 1X Detection Antibody Cocktail as follow:
   For running only **1 slide**: Dilute 150 µL of 10X Detection Antibody Cocktail with 1350 µL of Array Diluent Buffer.
   For running **2 slides**: Dilute 300 µL of 10X Detection Antibody Cocktail with 2700 µL of Array Diluent Buffer. *Keep on ice.
4. Prepare 1X HRP-linked Streptavidin as follow:
   For running only **1 slide**: Dilute 150 µL of 10X HRP-linked Streptavidin with 1350 µL of Array Diluent Buffer.
   For running **2 slides**: Dilute 300 µL of 10X HRP linked Streptavidin with 2700 µL of Array Diluent Buffer. *Keep on ice.
5. Affix the multi-well gasket to the glass slide (see figure at right):
   a. Place the multi-well gasket face-down on the benchtop (the silicone layer should be facing up). Remove the protective plastic film.
   b. Carefully place the glass slide on top of the multi-well gasket with the nitrocellulose pads facing down while aligning the pads with the openings in the gasket. The orientation line should appear in the upper left hand corner when the slide is oriented vertically.
   c. Insert the metal clip into the groove in the gasket and rotate the clip into the locked position. Ensure that the clip is on the same side as the orientation line on the slide.

Note: one of the clips has a small dot etched onto the upper rib to assist with pad designation (see slide assembly photos).

   d. Slide the clip into place.
   e. Snap the second metal clip to the other side of the assembly in the same manner and slide into place.
   f. The assembled array is ready to use.
6. Add 100 µl Array Blocking Buffer to each well and cover with sealing tape. Incubate for 15 minutes at room temperature on an orbital shaker.
   Note: Do not allow the pads to dry out at any time during the assay.
7. Decant Array Blocking Buffer by gently flicking out the liquid into a sink or other appropriate waste receptacle. Add 50 -75 µl diluted lysate to each well and cover with sealing tape. Incubate for 2 hours at room temp (or overnight at 4°C) on an orbital shaker.
8. Decant well contents by gently flicking out the liquid into a sink or other appropriate waste receptacle. Add 100 µl (1X) Array Wash Buffer to each well and incubate for 5 minutes at room temperature on an orbital shaker. Repeat three more times. Decant well contents.
9. Add 75 µl (1X) Detection Antibody Cocktail to each well and cover with sealing tape. Incubate for 1 hour at room temperature on an orbital shaker.
10. Wash 4 X 5 minutes with 100 µl (1X) Array Wash Buffer as in step 8.
11. Add 75 µl (1X) HRP-linked Streptavidin to each well and cover with sealing tape. Incubate for 30 minutes at room temperature on an orbital shaker.
12. Wash 4 X 5 minutes with 100 µl (1X) Array Wash Buffer as in step 8.
13. Remove multi-well gasket by pulling the bottom of the metal clips away from the center of the slide, then peeling the slide and gasket apart.
14. Place the slide face up in a plastic dish (a clean pipette tip box cover works well). Wash briefly with 10 ml (1X) Array Wash Buffer.
15. Dilute and combine LumiGLO® and Peroxide reagents immediately before use (to make 10 ml of a 1X solution, combine 9 ml deionized water with 0.5 ml of 20X LumiGLO® and 0.5 ml of 20X Peroxide). Note for Kodak Biomax film users: This dilution of LumiGlo®/Peroxide may necessitate very short exposure times (2-3 seconds) for some targets. For more convenient exposure times (20-30 seconds) add 20 ml of deionized water to the 10 ml LumiGlo®/Peroxide mix to make a 3 fold more diluted chemiluminescent reagent.
16. Decant Array Wash Buffer and cover slide with LumiGLO®/Peroxide reagent.
17. Transfer slide to chemiluminescent development folder, ensuring that it is still covered by LumiGLO®/ Peroxide reagent (add a small amount on top of the slide).
18. Immediately capture an image of the slide using a digital imaging system capable of detecting chemiluminescent signals. If desired, quantify spot intensities using commercially available array image analysis software. Alternatively, chemiluminescent film may be used. Expose film for 2-30 seconds using even and light pressure on the top of the development cassette (do not fasten the cassette clamps) to avoid squeezing out the LumiGLO®/ Peroxide reagent. Develop the film using an automated film developer.
    Note: If both slides are being used, it is not recommended to expose them simultaneously in the same development cassette. In this case, leave the second slide in the wash buffer (step 12) while proceeding with steps 13-18 using the first slide. After the first slide is finished, proceed with steps 13-18 using the second slide and freshly diluted LumiGLO®/Peroxide reagent.

LumiGLO® is a registered trademark of Kirkegaard & Perry Laboratories.



MSDS

Material Safety Data Sheet (MSDS) for PathScan® Intracellular Signaling Antibody Array Kit

Product name: PathScan® Intracellular Signaling Antibody Array Kit
Product Catalog: 7744, 7323 Kits
Manufacturer Supplier: Cell Signaling Technology
3 Trask Lane
Danvers, MA 01923 USA
978-867-2300 TEL
978-867-2400 FAX
978-578-6737 EMERGENCY TEL

II. Composition/Information:

Substance Name: PathScan® Intracellular Signaling Antibody Array Kit
CAS#: None
This product is For Research Use Only. According to 29 CFR 1910.1200(d), mixtures with hazardous ingredients at less than <1% and carcinogens at less than < 0.1% are considered non-hazardous. Please refer to the individual material safety data sheets for hazard information specific to kit components.

- Array Slides MSDS
- PathScan® Sandwich ELISA Lysis Buffer (1X) (CST#7018) MSDS
- Array Blocking Buffer MSDS
- Array Diluent Buffer MSDS
- Array Wash Buffer MSDS
- Detection Antibody Cocktail MSDS
- HRP-linked Streptavidin MSDS (Kit 7323 only)
- DyLight 680®-linked Steptavidin MSDS (Kit 7744 only)
- 20X LumiGLO & 20X Peroxide (CST#7003) MSDS

III. Hazard Identification:

Emergency Overview:
Not considered hazardous.
Not expected to produce significant adverse health effects when the recommended instructions for use are followed. No known significant effects or critical hazards.

IV. First Aid Measures:

Inhalation: Remove to fresh air. If breathing is difficult, get medical attention.
Ingestion: If person is conscious, wash out mouth with water. Get medical attention.
Skin exposure: Wash skin with soap and water. If irritation develops or persists, get medical attention.
Eye exposure: Immediately flush eyes water for at least 15 minutes. Get medical attention.

V. Fire Fighting Measures:

Flash Point: Not applicable.
Autoignition Temperature: Not applicable.
Explosion: Not applicable.
Fire extinguishing media: Water spray, dry chemical, alcohol foam, or carbon dioxide.
Firefighting: Wear protective clothing and self-contained breathing apparatus to prevent contact with skin and eyes.
Specific Hazard: None.

VI. Accidental Release Measures:

Wear appropriate personal protective equipment as indicated in Section VIII. Absorb liquid with an absorbent material. Transfer contaminated absorbent to a closed chemical waste container for disposal. Wash spill site after material has been picked up for disposal.

VII. Handling And Storage:

Storage: Store kit in tightly closed container at 4°C.

VIII - XIII.

Refer to individual MSDS for kit components for Sections 8-13 information: Exposure Controls/Personal Protection, Physical and Chemical Properties, Stability and Reactivity, Toxicological Information, Ecological information, Disposal Considerations.

XIV. Transport Information:

DOT: Proper Shipping Name: None.
This substance is considered Non-Hazardous for transport.

IATA: Proper Shipping Name: None.
This substance is considered Non-Hazardous for air transport.

XV. Regulatory Information:

EU Regulations/Classifications: Xi. Irritant.
Risk Phrases: Irritant. Irritating to eyes and skin. Harmful if swallowed.
Safety Phrases: In case of contact wash with water and seek medical attention.
US Regulatory Information: Irritant.

XVI. Other Information:

This product is not intended for use in humans. To the best of our knowledge, this document is accurate. It is intended to serve as a guide for safe use of this product in a laboratory setting by experienced personnel. The burden of safe use of this material rests entirely with the user. The above information is believed to be accurate but is not necessarily all-inclusive and shall be used only as a guide. Cell Signaling Technology, Inc., shall not be held liable for any damage resulting from the handling of or from contact with the above product.