Store at 4°C

#7137

# PathScan® Signaling Nodes I 4-Plex Array Kit

☑ 1 Kit
(96 assays)

Cell Signaling TECHNOLOGY®

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**This product is for *in vitro* research use only and is not intended for use in humans or animals.**
**This product is not intended for use as a therapeutic or in diagnostic procedures.**

**Introduction:** CST's PathScan® Signaling Nodes I 4-Plex Array Kit is based upon the sandwich ELISA principle. Rather than one immobilized antibody, these PathScan® 4-Plex Array Kits contain four different antibodies in each well of a 96-well plate. This allows the researcher to measure four different target proteins from a single well simultaneously. In addition, the incorporation of multiple phospho-specific antibody pairs allows the researcher to measure the activation state of important signaling nodes. The PathScan® Signaling Nodes I 4-Plex Array Kit allows the detection of: **Phospho-p38α MAPK (Thr180/Tyr182); Phospho-S6 Ribosomal Protein (Ser235/236); Phospho-p44 MAPK (Thr202/Tyr204); and Total Akt1** protein.

Briefly, 4 capture antibodies with distinct target specificity have been spotted onto the bottom of each well of a microwell plate. After incubation with cell lysates, the spotted antibodies capture the target proteins. Following extensive washing, a mixture of detection antibodies is added to detect the captured target proteins. An HRP-linked secondary solution is then used to recognize the bound detection antibodies. Chemiluminescent HRP substrate is used to produce luminescent signal.

| Products Included | Quantity |
|---|---|
| Microtiter well plate with four capture antibodies per well | 96 well plate |
| Sample diluent buffer | 10 ml |
| 20X Wash buffer | 50 ml |
| Detection antibody mix | 5.5 ml |
| Streptavidin-HRP solution | 5.5 ml |
| Sealing tape | 2 sheets |
| Chemiluminescent substrate | 5.5 ml |
| 10X Cell Lysis Buffer #9803 | 10 ml |

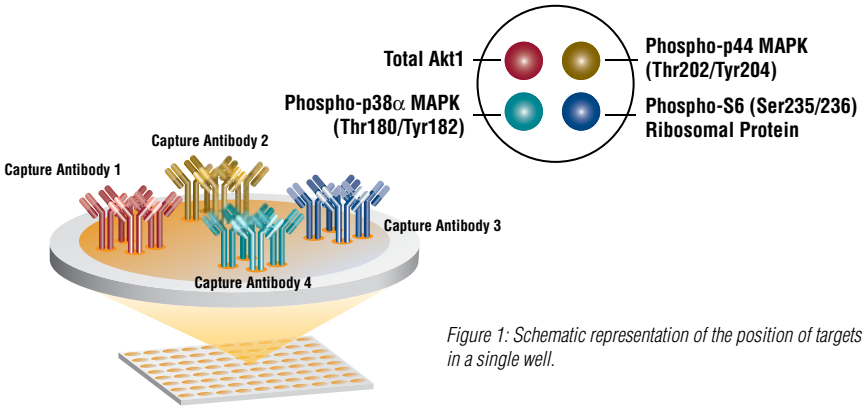


*Figure 1: Schematic representation of the position of targets in a single well.*

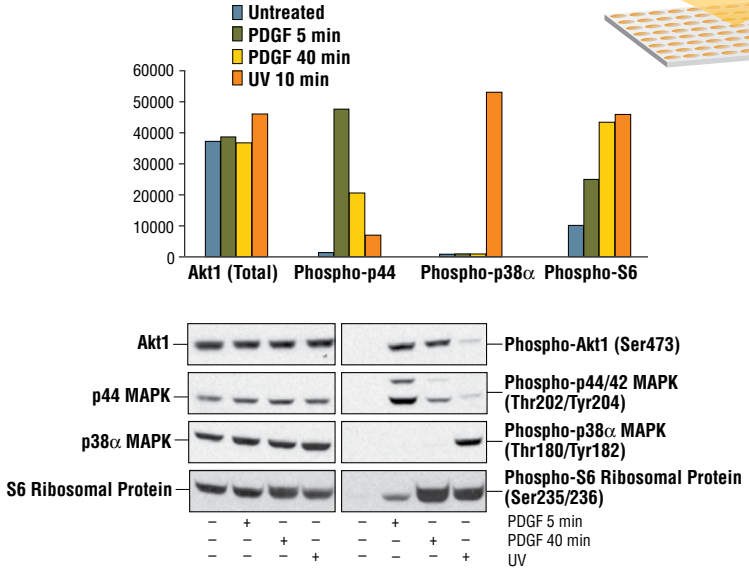


*Figure 2: NIH/3T3 cells were starved and stimulated with PDGF (100 ng/ml) for 5 or 40 minutes at 37°C. For treatment with UV, exponentially growing NIH/3T3 cells were irradiated without serum starvaiton. Lysates were prepared and analyzed using the PathScan® Signaling Nodes 1 4-Plex Array Kit. Luminescent signals were acquired using a Quansys Biosciences Imager. Spot intensity was quantified using Q-View™ software. The bar graph shows measurements of four different target proteins. Corresponding Western blots obtained with the same cell lysates are shown at the bottom.*

**Specificity/Sensitivity:** PathScan® Signaling Nodes I 4-Plex Array Kit detects endogenous levels of all target proteins. As shown in Figure 2, stimulation of NIH/3T3 cells with PDGF promotes phosphorylation of S6 ribosomal protein at Ser235/236, p44 MAP kinase at Thr202/Tyr204 while p38α phosphorylation levels remain unaffected. Conversely, stimulation with UV induces phosphorylation of p38α at Thr180/Tyr182 and S6 ribosomal protein at Ser235/236, while not affecting phosphorylation of p44 MAP kinase at Thr202/Tyr204. Levels of total Akt1 remain unchanged with all treatments.

**Species Cross-Reactivity: H, M**

**Required Equipment:**
Quansys Imager and Q-View Plus™ software (Quansys Biosciences, UT; www.quansysbio.com) are recommended for this assay kit for acquistion of the luminescent signal and quantification of spot intensity, respectively. Alternative imagers compatible with this array product are: Alpha Innotech Fluorchem HD2 or SP; Bio-Rad Versa Doc 4000 or XRS; Fujifilm LAS-3000.

QUANSYS BIOSCIENCES

*PathScan® 4-Plex Array Kit co-developed by Cell Signaling Technology® (CST) and Quansys Biosciences*

*Antibodies provided by CST. Platform provided by Quansys Biosciences.*

**Applications Key:** **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E**—ELISA

**Species Cross-Reactivity Key:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebra fish **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.

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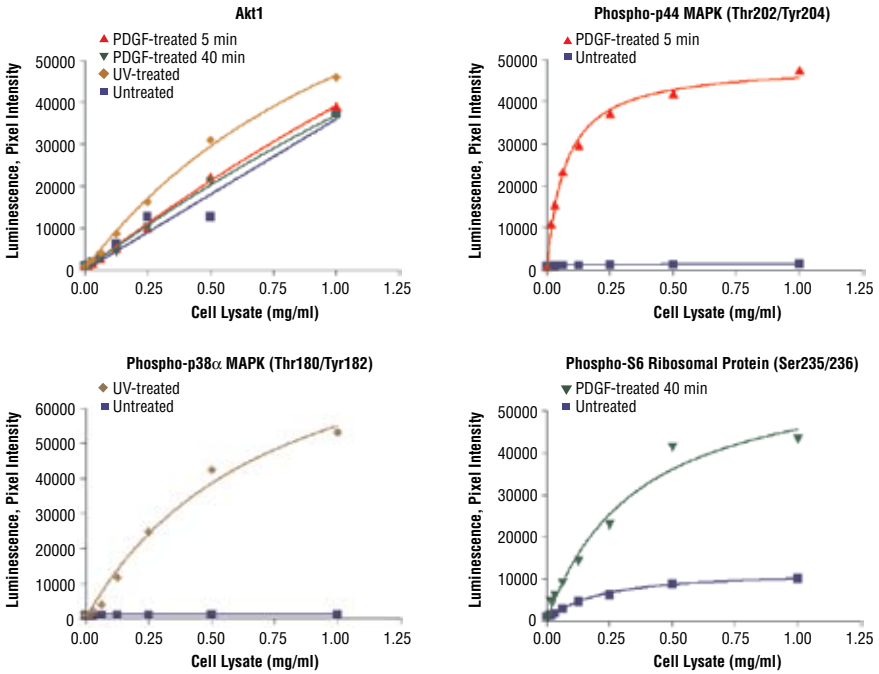


*Figure 3: Cell extracts from unstimulated or stimulated NIH/3T3 cells were applied to wells at increasing total protein concentrations and analyzed. Luminescent signals were acquired and spot intensity quantified. The graphs show the relationship between the total protein concentration and the resulting strength of the luminescent signal for each target.*

**Companion Products:**

PathScan® Total Akt1 Sandwich ELISA Kit #7170

PathScan® Phospho-p44 MAPK (Thr202/Tyr204) Sandwich ELISA Kit #7315

PathScan® Phospho-S6 Ribosomal Protein (Ser235/236) Sandwich ELISA Kit #7205

PathScan® Phospho-p38α MAPK (Thr180/Tyr182) Sandwich ELISA Kit #7140

Cell Lysis Buffer (10X) #9803

**Background:** Cells must respond in an appropriate fashion to many complex signaling events. Extracellular signaling cues are organized into well defined signal transduction modules that control fundamental cellular behavior. Two prominent signaling modules that are among the best characterized are the p44/42 MAP kinase (ERK MAPK) and p38 MAPK. The p44/42 MAPK is activated by a wide variety of extracellular signals including growth and neurotrophic factors, cytokines, hormones and neurotransmitters. p44/42 MAPK activation occurs through phosphorylation of threonine and tyrosine at the sequence T*EY* by a dual specificity kinase called MAP kinase kinase (MEK). p38 MAPK is dually phosphorylated in response to pro-inflammatory cytokines or cellular stress. These signaling modules are central controllers of fundamental cellular behavior such as growth, proliferation, movement, and response to stress. Growth factor stimulation results in phosphorylation of S6 ribosomal protein on Ser235/236, leading to an increase in protein synthesis and cell cycle progression. Phosphorylation levels of critical molecular switches such as MAPKs therefore serve as a reliable indicator of the activation state of the entire signaling module. The profiling of phosphorylation events using phospho-specific antibodies is now widely used to investigate diagnostic pathology (1,2). While profiling of protein phosphorylation events was shown to predict the progression of a tumor to a more invasive stage (3), it has been observed that the ratio between p44/42 MAPK and p38 MAPK may predict whether tumor cells will proliferate or enter a state of dormancy *in vivo* (4). PathScan® Signaling Nodes I 4-Plex Array Kit provides the researcher with means to profile numerous chemical compounds and obtain in-cell relative potency (5).

**Background References:**

(1) Danna, E.A. and Nolan, G.P. (2006) *Curr Opin Chem Biol* 10, 20–7.

(2) Irish, J.M. et al. (2006) *Nat Rev Cancer* 6, 146–55.

(3) Sheehan, K.M. et al. (2008) *Oncogene* 27, 323–31.

(4) Aguirre-Ghiso, J.A. et al. (2003) *Cancer Res* 63, 1684–95.

(5) Gechtman Z. (2006) *American Drug Discovery* 1:1, 44–52.

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# PathScan® 4-Plex Array Kit Protocol

## A Reagent Preparation

1. Prior to running the assay bring the 96-well plate to room temperature.
2. Dilute the 20X Wash Buffer to a 1X solution with deionized water. Mix thoroughly. Store at 4°C until ready to use.
3. Thaw the 1X Cell Lysis Buffer (#9803) and mix gently but thoroughly. Supplement the cell lysis buffer with PMSF to 1 mM final concentration prior to performing the assay (other protease inhibitors may be added).

## B Preparing Cell Lysates

1. Place cells on ice and wash once with ice-cold PBS.
2. Scrape cells in 0.5 ml of ice-cold cell lysis buffer (per 10 -15 cm plate), gently mix and incubate for 5 minutes on ice.
3. Sonicate cells on ice using medium strength output by creating 4 sonic pulses, 10 seconds each, with 30- 60 sec. intervals.
4. Microcentrifuge for 10 minutes at 4°C to pellet the insoluble material and transfer the supernatant to a new tube. The resultant supernatant is the cell lysate that is ready to be analyzed. The cell lysate can also be stored at -80°C in single-use aliquots for future use.
5. Determine protein concentration of the cell lysates and make appropriate dilutions using the sample diluent buffer. Recommended range of final total protein concentration to be applied onto 96-well plates is: 0.1 – 0.5 mg/ml. (Use the dose response curves that appear in the datasheet as a reference). As a guideline, the total protein concentration of a typical cell lysate generated from adherent cells grown on a 15 cm plate to 80-90 % confluency will be in the range of 0.5 – 2.5 mg/ml.

## C Test Procedure

1. Add 50 µl of diluted cell extract into each well.
2. Cover the 96-well plate with plate seal and incubate on a shaker (~120 rpm) for 1 hour at room temperature.
3. Wash the plate 3 times with Wash Buffer, 200-400 µl per well.
4. Add 50 µl of the Detection mix into each well.
5. Reseal the plate with the provided seal and incubate on a shaker (~120 rpm) for 1 hour at room temperature.
6. Wash the plate 3 times with Wash Buffer.
7. Add 50 µl of the Strepavidin-HRP solution into each well.
8. Reseal the plate with a plate seal and incubate on a shaker (~120 rpm) for 15 min. at room temperature.
9. Prepare the chemiluminescent substrate by mixing the Substrate bottle A with Substrate bottle B and set aside at room temperature.
10. Wash the plate 6 times with Wash Buffer.
11. Add 50 µl of the mixed chemiluminescent substrate into each well.
12. Acquire Luminescent signal by imaging the plate using a Quansys imager (Quansys Biosciences, UT; www.quansysbio.com; Cat # 104450GR; www.quansysbio.com/support/imagers/quansys.html). Spot intensity can be quantified using Q-View™ or Q-View Plus™ softwares from Quansys Biosciences. Alternative imagers compatible with this array product are: Alpha Innotech Fluorchem HD2 or SP; Bio-Rad Versa Doc 4000 or XRS; or Fujifilm LAS-3000.

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