

#7327C Store at 4°C

PathScan® Phospho-PRAS40 (Thr246) Sandwich ELISA Kit



✓ 1 Kit
(96 assays)

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rev. 08/01/17

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

Species Cross-Reactivity: H, M

Description: The PathScan® Phospho-PRAS40 (Thr246) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of PRAS40 when phosphorylated at Thr246. A PRAS40 rabbit antibody has been coated onto the microwells. After incubation with cell lysates, PRAS40 protein (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, a biotinylated phospho-PRAS40 (Thr246) rabbit detection antibody is added to detect captured phospho-PRAS40 (Thr246) protein. HRP-linked streptavidin is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of the absorbance for the developed color is proportional to the quantity of PRAS40 phosphorylated at Thr246.

Specificity/Sensitivity: PathScan® Phospho-PRAS40 (Thr246) Sandwich ELISA Kit detects endogenous levels of PRAS40 protein when phosphorylated at Thr246 as shown in Figure 1. Kit sensitivity is shown in Figure 2.

Background: Many growth factors and hormones induce the phosphoinositide 3-kinase signaling pathway, which results in the activation of downstream effector proteins such as the serine/threonine kinase Akt (1,2). One known Akt substrate is a 40 kDa, proline-rich protein (PRAS40) that binds to 14-3-3 protein (2). PRAS40 also binds mTOR to transduce Akt signals to the mTOR complex. Inhibition of mTOR signaling stimulates PRAS40 binding to mTOR, which in turn inhibits mTOR activity (3). PRAS40 interacts with Raptor in mTOR complex 1 (mTORC1) in insulin-deprived cells and inhibits the activation of the mTORC1 pathway mediated by the cell cycle protein Rheb. Phosphorylation of PRAS40 by Akt at Thr246 relieves PRAS40 inhibition of mTORC1 (4). mTORC1 in turn phosphorylates PRAS40 at Ser183 (5).

Background References:

- (1) Cantley, L.C. (2002) *Science* 296, 1655-7.
- (2) Kovacina, K.S. et al. (2003) *J Biol Chem* 278, 10189-94.
- (3) Vander Haar, E. et al. (2007) *Nat Cell Biol* 9, 316-23.
- (4) Sancak, Y. et al. (2007) *Mol Cell* 25, 903-15.
- (5) Oshiro, N. et al. (2007) *J Biol Chem* 282, 20329-39.

| Product Includes | Item # | Kit Quantity | Color | Storage Temp |
|---|--------|--------------|---------------------|--------------|
| PRAS40 Rabbit mAb Coated Microwells* | 82334 | 96 tests | | 4°C |
| Phospho-PRAS40 (Thr246) Rabbit Detection mAb (Biotinylated) | 8196 | 1 each | Green (Lyophilized) | 4°C |
| HRP-linked Streptavidin (ELISA Formulated) | 11805 | 1 each | Red (Lyophilized) | 4°C |
| Detection Antibody Diluent | 13339 | 11 ml | Green | 4°C |
| HRP Diluent | 13515 | 11 ml | Red | 4°C |
| TMB Substrate | 7004 | 11 ml | | 4°C |
| STOP Solution | 7002 | 11 ml | | 4°C |
| Sealing Tape | 54503 | 2 sheets | | 4°C |
| ELISA Wash Buffer (20X) | 9801 | 25 ml | | 4°C |
| ELISA Sample Diluent | 11083 | 25 ml | Blue | 4°C |
| Cell Lysis Buffer (10X) | 9803 | 15 ml | | -20°C |

*12 8-well modules – Each module is designed to break apart for 8 tests.

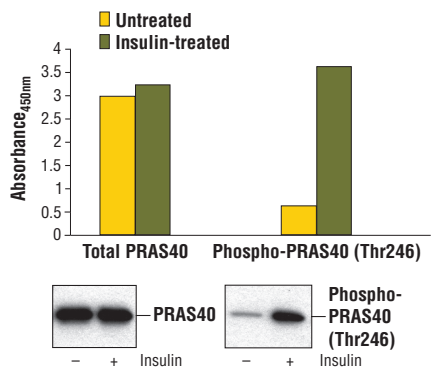


Figure 1. Treatment of HeLa cells with insulin stimulates phosphorylation of PRAS40 at Thr246, detected by the PathScan® Phospho-PRAS40 (Thr246) Sandwich ELISA Kit, but does not affect the levels of total PRAS40 detected by PathScan® Total PRAS40 Sandwich ELISA Kit #7331. HeLa cells (80-90% confluent) were treated with 1.7 μM insulin for 15 minutes and lysed. The absorbance readings at 450 nm are shown in the top figure, while the corresponding western blots using PRAS40 (D23C7) XP® Rabbit mAb #2691 (left panel) or Phospho-PRAS40 (Thr246) (C77D7) Rabbit mAb #2997 (right panel) are shown in the bottom figure.

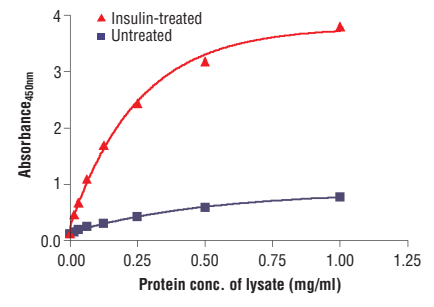


Figure 2. The relationship between the protein concentration of lysates from untreated and insulin-treated HeLa cells and the absorbance at 450 nm using the PathScan® Phospho-PRAS40 (Thr246) Sandwich ELISA Kit is shown.

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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 Ce—C. elegans Hr—horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

U.S. Patent No. 5,675,063

PathScan® Sandwich ELISA Protocol (for kits with Lyophilized Antibodies)

A Solutions and Reagents

NOTE: Prepare solutions with purified water.

- Microwell strips:** Bring all to room temperature before use.
- Detection Antibody:** Supplied lyophilized as a green colored cake or powder. Add 1.0 ml of Detection Antibody Diluent (green solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 1.0 ml volume of reconstituted Detection Antibody to 10.0 ml of Detection Antibody Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
- HRP-Linked Antibody*:** Supplied lyophilized as a red colored cake or powder. Add 1.0 ml of HRP Diluent (red solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 1.0 ml volume of reconstituted HRP-Linked Antibody to 10.0 ml of HRP Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
- Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody (11 ml provided).
- HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (11 ml provided).
- Sample Diluent:** Blue colored diluent provided for dilution of cell lysates.
- 1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in purified water.
- Cell Lysis Buffer:** 10X Cell Lysis Buffer #9803: This buffer can be stored at 4°C for short-term use (1–2 weeks). Recommended: Add 1 mM phenylmethyl-sulfonyl fluoride (PMSF) immediately before use.
- TMB Substrate** (#7004).
- STOP Solution** (#7002).

*Note: Some PathScan® ELISA Kits may include HRP-Linked Streptavidin in place of HRP-Linked Antibody.

B Preparing Cell Lysates

For adherent cells.

- Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
- Remove media and rinse cells once with ice-cold 1X PBS.
- 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.
- Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
- Sonicate lysates on ice.
- Microcentrifuge for 10 min (14,000 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

- Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 × 10⁶ viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
- Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
- Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X Cell Lysis Buffer plus 1 mM PMSF.
- Sonicate lysates on ice.
- Microcentrifuge for 10 min (14,000 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 Test Procedure

- After the microwell strips have reached room temperature, break off the required number of microwells. Place the microwells in the strip holder. Unused microwells must be resealed and stored at 4°C immediately.
- Cell lysates can be undiluted or diluted with Sample Diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color). Individual datasheets for each kit provide a sensitivity curve that serves as a reference for selection of an appropriate starting lysate concentration. The sensitivity curve shows typical kit assay results across a range of lysate concentration points.
- Add 100 µl of each undiluted or diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hr at 37°C. Alternatively, the plate can be incubated overnight at 4°C.
- Gently remove the tape and wash wells:
 - Discard plate contents into a receptacle.
 - Wash 4 times with 1X Wash Buffer, 200 µl each time for each well.
 - For each wash, strike plates on fresh towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
 - Clean the underside of all wells with a lint-free tissue.
- Add 100 µl of reconstituted Detection Antibody (green color) to each well (refer to Section A, Step 2). Seal with tape and incubate the plate at 37°C for 1 hr.
- Repeat wash procedure (Section C, Step 4).
- Add 100 µl of reconstituted HRP-Linked secondary antibody (red color) to each well (refer to Section A, Step 3). Seal with tape and incubate the plate for 30 min at 37°C.
- Repeat wash procedure (Section C, Step 4).
- Add 100 µl of TMB Substrate to each well. Seal with tape and incubate the plate for 10 min at 37°C or 30 min at 25°C.
- Add 100 µl of STOP Solution to each well. Shake gently for a few seconds.

NOTE: Initial color of positive reaction is blue, which changes to yellow upon addition of STOP Solution.

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