#7300C Store at 4°C

PathScan® Phospho-Stat3 (Tyr705) Sandwich ELISA Kit

☑ 1 Kit
(96 assays)

Cell Signaling TECHNOLOGY®

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

Species Cross-Reactivity: H, M, R, Mk

Description: CST's PathScan® Phospho-Stat3 (Tyr705) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of Phospho-Stat3 (Tyr705) protein. A Stat3 rabbit monoclonal antibody has been coated onto the microwells. After incubation with cell lysates, both nonphospho- and phospho-Stat3 proteins are captured by the coated antibody. Following extensive washing, a phospho-Stat3 mouse monoclonal antibody is added to detect the captured phospho-Stat3 protein. HRP-linked anti-mouse antibody is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of optical density for this developed color is proportional to the quantity of phospho-Stat3 protein.

Specificity/Sensitivity: CST's PathScan® Phospho-Stat3 (Tyr705) Sandwich ELISA Kit detects endogenous levels of Phospho-Stat3 protein. Using this Sandwich ELISA Kit #7300, a significant induction of phospho-Stat3 can be detected in IFN-α-treated HeLa cells. However, the level of total Stat3 protein remains unchanged, as shown by Western analysis (figure 1). This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

Background: The Stat3 transcription factor is an important signaling molecule for many cytokines and growth factor receptors (1) and is required for murine fetal development (2). Stat3 is constitutively activated in a number of human tumors (3,4) and possesses oncogenic potential (5) and anti-apoptotic activities (3). Stat3 is activated by phosphorylation at Tyr705, which induces dimerization, nuclear translocation, and DNA binding (6,7). Transcriptional activation seems to be regulated by phosphorylation at Ser727 through the MAPK or mTOR pathways (8,9). Stat3 isoform expression appears to reflect biological function as the relative expression levels of Stat3α (86 kDa) and Stat3β (79 kDa) depend on cell type, ligand exposure, or cell maturation stage (10). It is notable that Stat3β lacks the serine phosphorylation site within the carboxy-terminal transcriptional activation domain (8).

Product Includes	Item #	Kit Quantity	Color	Storage Temp
Stat3 Rabbit mAb Coated Microwells*	40936	96 tests		4°C
Phospho-Stat3 (Tyr705) Mouse Detection mAb	13149	1 each	Green (Lyophilized)	4°C
Anti-mouse IgG, HRP-linked Antibody (ELISA Formulated)	13304	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.

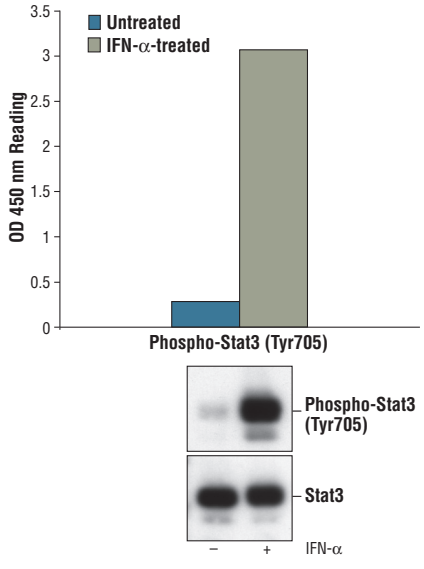

Figure 1: Treatment of HeLa cells with IFN-α stimulates phosphorylation of Stat3 at Tyr705, detected by PathScan® Phospho-Stat3 (Tyr705) Sandwich ELISA kit, #7300, but does not affect the level of total Stat3 protein, detected by a Stat3 antibody via Western analysis. OD450 readings are shown in the top figure, while the corresponding Western blot using Phospho-Stat3 (Tyr705) (3E2) Monoclonal Antibody #9138 or Stat3 antibody, is shown in the bottom figure.

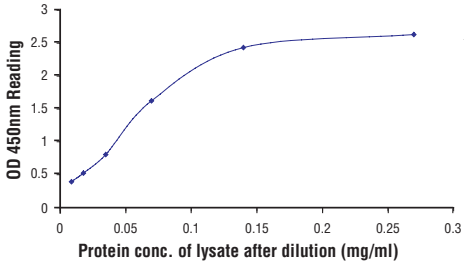

Figure 2: Linear relationship between protein concentration of lysates from IFN-α-treated HeLa cells and kit assay optical density readings. HeLa cells (75% confluence) were treated with IFN-α (100 ng/ml), and lysed after growth at 37°C for 10 min.

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

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.

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

A Solutions and Reagents

NOTE: Prepare solutions with purified water.

1. **Microwell strips:** Bring all to room temperature before use.
2. **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.
3. **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.
4. **Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody (11 ml provided).
5. **HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (11 ml provided).
6. **Sample Diluent:** Blue colored diluent provided for dilution of cell lysates.
7. **1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in purified water.
8. **Cell Lysis Buffer:** 10X Cell Lysis Buffer #9803 or 1X Cell Lysis Buffer #7018: This buffer can be stored at 4°C for short-term use (1–2 weeks). Recommended: Add 1 mM phenylmethylsulfonyl fluoride (PMSF) immediately before use.
9. **TMB Substrate** (#7004).
10. **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.

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 (~1200 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 (~1200 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 Test Procedure

1. 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.
2. 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.
3. 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.
4. Gently remove the tape and wash wells:
   a. Discard plate contents into a receptacle.
   b. Wash 4 times with 1X Wash Buffer, 200 µl each time for each well.
   c. 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.
   d. Clean the underside of all wells with a lint-free tissue.
5. 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.
6. Repeat wash procedure (Section C, Step 4).
7. 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.
8. Repeat wash procedure (Section C, Step 4).
9. 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.
10. 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.

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

Background References:

(1) Heim, M.H. (1999) *J. Recept. Signal Transduct. Res.* 19, 75-120.

(2) Takeda, K. et al. (1997) *Proc. Natl. Acad. Sci. USA* 94, 3801-3804.

(3) Catlett-Falcone, R. et al. (1999) *Immunity* 10, 105-115.

(4) Garcia, R. and Jove, R. (1998) *J. Biomed. Sci.* 5, 79-85.

(5) Bromberg, J.F. et al. (1999) *Cell* 98, 295-303.

(6) Darnell Jr., J.E. et al. (1994) *Science* 264, 1415-1421.

(7) Ihle, J.N. (1995) *Nature* 377, 591-594.

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(9) Yokogami, K. et al. (2000) *Curr. Biol.* 10, 47-50.

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