#7300 Store at 4°C

# PathScan® Phospho-Stat3 (Tyr705) Sandwich ELISA Kit

☑ 1 Kit (96 assays)

rev. 08/23/04

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

**Species Cross-Reactivity: H**

**Introduction:** 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 (7300–52F7*) 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 #9138* is added to detect the captured phospho-Stat3 protein. HRP-linked anti-mouse antibody #7076* 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.

*Antibodies in the kit are custom formulations specific to the kit.

**Companion Products:**

Phospho-Stat3 (Tyr705) (3E2) Mouse Monoclonal Antibody #9138

Anti-mouse IgG, HRP-linked Antibody #7076

Cell Lysis Buffer (10X) #9803

**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, shown by Western analysis using Stat3 rabbit monoclonal antibody (7300–52F7) (figure 1).

## Kit Includes

| Products | Volume | Solution Color |
|---|---|---|
| Stat3 (7300-52F7) Rabbit Monoclonal Ab Coated Microwells* | 96 tests | |
| Phospho-Stat3 (Tyr705) Detection Ab | 11 ml | green |
| Anti-mouse IgG HRP-Linked Ab | 11 ml | red |
| TMB Substrate | 11 ml | colorless |
| STOP Solution | 11 ml | colorless |
| Sealing Tape | 2 sheets | |
| 20X Wash Buffer | 25 ml | colorless |
| Sample Diluent | 25 ml | blue |
| 10X Cell Lysis Buffer #9803** | 15 ml | yellowish |

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

**Kit should be stored at 4°C wtih the exception of 10X Cell Lysis Buffer, which is stored at –20°C (packaged separately).

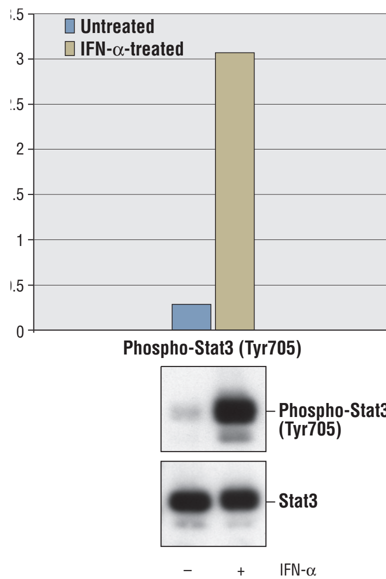


*Figure 1: Treatment of HeLa cells with IFN-α stimulates phosphorylation of Stat3 at Tyr705, detected by PathScan® Phospho-Stat3 (Tyr705) Sandwich ELISA kit, #7300, but does not affect the level of total Stat3 protein, detected by Stat3 rabbit monoclonal antibody (7300-52F7) via Western analysis. OD450 readings are shown in the top figure, while the corresponding Western blot using Phospho-Stat3 (Tyr705) (3E2) Monoclonal Antibody #9138 or Stat3 rabbit monoclonal antibody (7300-52F7), is shown in the bottom figure.*

© 2004 Cell Signaling Technology, Inc.
This product is for research use only and is not intended for use in humans.
Rabbit Monoclonals Produced Using Epitomics® Technology, U.S. Patent No. 5,675,063.

**Species Cross-Reactivity Key:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **X**—*Xenopus* **Z**—zebra fish **All**—all species expected

Cell Signaling TECHNOLOGY®

**Background:** Stat3 is a key signaling molecule for many cytokines and growth-factor receptors (1), and is required for murine fetal development (2). Additionally, 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, apparently via the MAPK or mTOR pathways (8,9). Stat3 isoform expression appears to reflect biological function: the relative expression levels of Stat3alpha (86 kDa) and Stat3β (79 kDa) depend on cell type, ligand exposure or maturation stage of the cells (10). It is notable that Stat3β lacks the serine phosphorylation site within the carboxy-terminal transcriptional activation domain (8).

**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, 3801–227.

(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.

(8) Wen, Z. et al. (1995) *Cell* 82, 241–250.


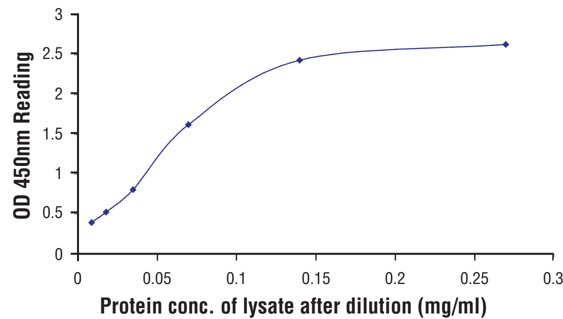


*Figure 2: Linear relationship between protein concentration of lysates from IFN-α-treated HeLa cells and kit assay optical density readings. HeLa cells (75% confluence) were treated with IFN-α (100 ng/ml), and lysed after growth at 37°C for 10 min.*

© 2004 Cell Signaling Technology, Inc.

Cell Signaling TECHNOLOGY®

# Sandwich ELISA Protocol

## A Reagent Preparation

**A1** Bring all microwell strips to room temperature before use.

**A2** Prepare 1X Wash Buffer using Milli-Q or equivalently purified water.

**A3** **1X Cell Lysis Buffer from CST #9803:** 20 mM Tris (pH7.5), 150 mM NaCl, 1 mM ethylene diaminetetraacetate (EDTA), 1 mM ethylene glycol-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerolphosphate, 1 mM $Na_3VO_4$, 1µg/ml leupeptin. This buffer can be stored at 4°C for short-term use (1–2 weeks).

## B Preparing Cell Lysates

**B1** Aspirate media. Treat cells by adding fresh media containing regulator for desired time.

**B2** To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.

**B3** Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenylmethylsulfonyl fluoride (PMSF) to each plate (10 $cm^2$) and incubate the plate on ice for 5 minutes.

**B4** Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.

**B5** Sonicate lysates on ice.

**B6** Microcentrifuge for 10 minutes at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Aliquot and store at –80°C.

## C Test Procedure

**C1** After the microwell strips have reached room temperature, break off the required numbered of microwells. Place the microwells in the strip holder. Unused microwells must be resealed and stored at 4°C immediately.

**C2** Add 100 µl of **Sample Diluent** (blue color) to a microcentrifuge tube. Transfer 100 µl of cell lysate into the tube and vortex for a few seconds. (Sample applied to the well can be diluted 1:1 when the suggested cell lysis buffer is used for cell extraction.) Figure 2 provides a reference in dilution factor for lysates and kit assay results.

**C3** Add 100 µl of each diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hours at 37°C. Alternatively, the plate can be incubated overnight at 4°C, which gives the best detection of target protein.

**C4** 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.

**C5** Add 100 µl of **Detection Antibody** (green color) to each well. Seal with tape and incubate the plate for 1 hour at 37°C.

**C6** Repeat wash procedure as in Step C4.

**C7** Add 100 µl of **HRP-Linked** secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 minutes at 37°C.

**C8** Repeat wash procedure as in Step C4.

**C9** Add 100 µl of **TMB Substrate** to each well. Seal with tape and incubate the plate for 10 minutes at 37°C or 30 minutes at 25°C.

**C10** 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.

**C11** Read results.

a. Visual Determination — Read within 30 minutes after adding STOP Solution.

b. Spectrophotometric Determination — Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 minutes after adding STOP Solution.

© 2004 Cell Signaling Technology, Inc.