Store at 4°C

#7113

PathScan® Phospho-Stat5 (Tyr694) Sandwich ELISA Kit

☑ 1 Kit
(96 assays)

Cell Signaling TECHNOLOGY®

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New 01/08

Species Cross-Reactivity: H

Introduction: CST's PathScan® Phospho-Stat5 (Tyr694) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of Stat5 when phosphorylated at Tyr694. A Phospho-Stat5 Mouse Antibody* has been coated onto the microwells. After incubation with cell lysates, phospho-Stat5 (Tyr694) is captured by the coated antibody. Following extensive washing, a Stat5 Rabbit Detection Antibody* is added to detect the captured phospho-Stat5 protein. Anti-rabbit IgG, HRP-linked antibody #7074* is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of absorbance for this developed color is proportional to the quantity of Stat5 phosphorylated at Tyr694.

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

Companion Products:

Phospho-Stat5 (Tyr694) Antibody #9351

Phospho-Stat5 (Tyr694) (C11C5) Rabbit mAb #9359

Phospho-Stat5(Tyr694) (14H2) Mouse mAb #9356

Stat5 (3H7) Rabbit mAb #9358

Stat5 Antibody #9363

Stat5 Antibody #9310

Anti-rabbit IgG, HRP-linked Antibody #7074

Cell Lysis Buffer (10X) #9803

TMB Substrate #7004

STOP Solution #7002

Phosphate Buffered Saline (PBS-20X) #9808

Phosphate Buffered Saline with Tween 20 (PBST-20X) #9809

Specificity/Sensitivity: CST's PathScan® Phospho-Stat5 (Tyr694) Sandwich ELISA Kit #7113 detects endogenous levels of phospho-Stat5 when phosphorylated at Tyr694. As shown in Figure 1, a significant induction of Stat5 phosphorylation at Tyr694 can be detected in EGF-treated HeLa cells using the Phospho-Stat5 (Tyr694) Sandwich ELISA Kit #7113. The level of total Stat5 detected by Western analysis remains unchanged.

Products Included	Volume	Solution Color
Phospho-Stat5 (Tyr694) Mouse Antibody Coated Microwells*	96 tests	
Stat5 Rabbit Detection Antibody	11 ml	green
Anti-Rabbit IgG HRP-Linked Antibody	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 with the exception of 10X Cell Lysis Buffer, which is stored at –20°C (packaged separately).

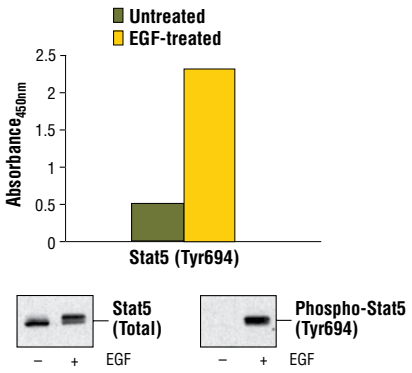

Figure 1. Treatment of HeLa cells with EGF stimulates phosphorylation of Stat5 at Tyr694, detected by the PathScan® Phospho-Stat5 (Tyr694) Sandwich ELISA Kit #7113, but does not affect the level of total Stat5 protein detected by Western analysis. HeLa cells (80-90% confluent) were starved overnight and treated with 100 ng/mL EGF for 40 minutes at 37°C. The absorbance readings at 450 nm are shown in the top figure, while the corresponding Western blots, using Stat5 (3H7) Rabbit mAb #9358 (left panel) or Phospho-Stat5 (Tyr694) (14H2) Mouse mAb #9356 (right panel), are shown in the bottom figure.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **IC**—Immunocytochemistry **IF**—Immunofluorescence **F**—Flow cytometry **E**—ELISA **D**—DELFIA®

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

Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Background: Stat5 is activated in response to a wide variety of ligands including IL-2, GM-CSF, growth hormone and prolactin. Phosphorylation at Tyr694 is obligatory for Stat5 activation (1,2). This phosphorylation is mediated by Src upon erythropoietin stimulation (3). Stat5 has been found to be constitutively active in some leukemic cell types (4). Phosphorylated Stat5 is found in some endothelial cells treated with IL-3, which suggests its involvement in angiogenesis and cell motility (5). Stat5α and β are independently regulated and activated in various cell types. For instance, both isoforms are activated in response to IFN-α in B cells, but only Stat5α is phosphorylated in response to IFN-α in HeLa cells (6).

Background References:

(1) Gouilleux, F. et al. (1994) *EMBO J.* 13, 4361–4369.

(2) Wakao, H. et al. (1994) *EMBO J.* 13, 2182–2191.

(3) Okutani, Y. et al. (2001) *Oncogene* 20, 6643–6650.

(4) Demoulin, J.B. et al. (1999) *J. Biol. Chem.* 274, 25855–25861.

(5) Dentelli, P. et al. (1999) *J. Immunol.* 163, 2151–2159.

(6) Meinke, A. et al. (1996) *Mol. Cell. Biol.* 16, 6937–6944.

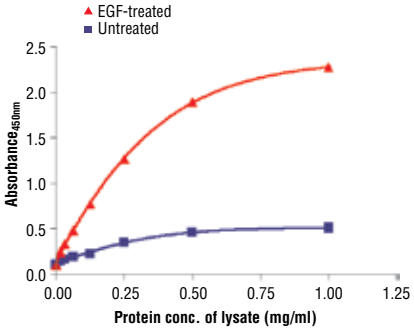

Figure 2. The relationship between the protein concentration of the lysate from untreated and EGF-treated HeLa cells and the absorbance at 450 nm is shown.

Sandwich ELISA Protocol

A Reagent Preparation

1. Bring all microwell strips to room temperature before use.
2. Prepare 1X Wash Buffer by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in Milli-Q or equivalently purified water.
3. **1X Cell Lysis Buffer from CST #9803:** 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM ethylene diamine tetraacetate (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-glycerophosphate, 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

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenylmethylsulfonyl fluoride (PMSF) to each plate (10 cm in diameter) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 minutes 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. Add 100 µl of Sample Diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color) to a microcentrifuge tube. Transfer 100 µl of cell lysate into the tube and vortex for a few seconds. Generally, sample applied to the well can be diluted 1:1 when the suggested cell lysis buffer is used for cell extraction. Individual data sheets for each kit provide information regarding an appropriate dilution factor for lysates and kit assay results. However, dilution factors need to be titrated when specific cell lysates are used.
3. 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.
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 Detection Antibody (green color) to each well. Seal with tape and incubate the plate for 1 hour at 37°C.
6. Repeat wash procedure as in Step 4.
7. 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.
8. Repeat wash procedure as in Step 4.
9. 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.
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 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.