

PathScan® Phospho-Stat5 (Tyr694) Sandwich ELISA Antibody Pair



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Species Cross Reactivity:
H

UniProt ID:
#P42229, #P51692

Entrez-Gene ID:
#6776, #6777

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Description

CST's PathScan® Phospho-Stat5 (Tyr694) Sandwich ELISA Antibody Pair is being offered as an economical alternative to our PathScan® Phospho-Stat5 (Tyr694) Sandwich ELISA Kit #7113. Capture and detection antibodies (100X stocks) and an HRP-conjugated secondary antibody (1000X stock) are supplied. Sufficient reagents are supplied for 4 x 96 well ELISAs. The Phospho-Stat5 (Tyr694) Mouse Capture Antibody is coated in PBS overnight in a 96 well microplate. After blocking, cell lysate is added followed by a Stat5 Rabbit Detection Antibody and an HRP-conjugated Anti-Rabbit IgG Antibody. HRP substrate (TMB) is added for color development. The magnitude of the absorbance for this developed color is proportional to the quantity of Stat5 phosphorylated at Tyr694.

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

Reagents Not Supplied

Phosphate Buffered Saline (PBS-20X) #9808 Phosphate Buffered Saline with Tween-20 (PBST-20X) #9809 Cell Lysis Buffer (10X) #9803 TMB Substrate #7004 STOP Solution #7002 Blocking Buffer: 1X PBS/0.05% Tween-20, 1% BSA 96 Well Microplates** Microplate Reader ** Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592) and Corning® 96 Well EIA/RIA Easy Wash™ Clear Flat Bottom Polystyrene High Bind Microplates (#3369).

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 is 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). Stat5a and Stat5b are independently regulated and activated in various cell types. For instance, interferon treatment predominantly activates Stat5a in U-937 cells and Stat5b in HeLa cells (6).

Background References

1. Gouilleux, F. et al. (1994) *EMBO J* 13, 4361-9.
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3. Okutani, Y. et al. (2001) *Oncogene* 20, 6643-50.
4. Demoulin, J.B. et al. (1999) *J Biol Chem* 274, 25855-61.
5. Dentelli, P. et al. (1999) *J Immunol* 163, 2151-9.
6. Meinke, A. et al. (1996) *Mol Cell Biol* 16, 6937-44.

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#7281

PathScan[®] Phospho-Stat5 (Tyr694) Sandwich ELISA Antibody Pair

ELISA Antibody Pair

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH₂O, mix.
2. **Wash Buffer:** 1X PBS/0.05% Tween[®] 20, (20X PBST #9809).
3. **Blocking Buffer:** 1X PBS/0.05% Tween[®] 20, 1% BSA.
4. **1X Cell Lysis Buffer:** 10X Cell Lysis Buffer (#9803): To prepare 10 ml of 1X Cell Lysis Buffer, add 1 ml of 10X Cell Lysis Buffer to 9 ml of dH₂O, mix. Buffer can be stored at 4°C for short-term use (1-2 weeks).

Recommended: Add 1 mM phenylmethylsulfonyl fluoride (PMSF) (#8553) immediately before use.

5. **Bovine Serum Albumin (BSA):** (#9998).
6. **TMB Substrate:** (#7004).
7. **STOP Solution:** (#7002)

NOTE: Reagents should be made fresh daily.

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 (~1,200 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 (~1,200 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. Coating Procedure

1. Rinse microplate with 200 µl of dH₂O, discard liquid. Blot on paper towel to make sure wells are dry.
2. Dilute capture antibody 1:100 in 1X PBS. For a single 96 well plate, add 100 µl of capture antibody stock to 9.9 ml 1X PBS. Mix well and add 100 µl/well. Cover plate and incubate overnight at 4°C (17-20 hr).
3. **After overnight coating, gently uncover plate and wash wells:**
 1. Discard plate contents into a receptacle.
 2. Wash four times with wash buffer, 200 µl each time per well. For each wash, strike plates on fresh paper towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
 3. Clean the underside of all wells with a lint-free tissue.
4. Block plates. Add 150 µl of blocking buffer/well, cover plate, and incubate at 37°C for 2 hr.
5. After blocking, wash plate (Section C, Step 3). Plate is ready to use.

D. Test Procedure

1. Lysates can be used undiluted or diluted in blocking buffer. 100 μ l of lysate is added per well. Cover plate and incubate at 37°C for 2 hr.
2. Wash plate (Section C, Step 3).
3. Dilute detection antibody 1:100 in blocking buffer. For a single 96 well plate, add 100 μ l of detection antibody Stock to 9.9 ml of blocking buffer. Mix well and add 100 μ l/well. Cover plate and incubate at 37°C for 1 hr.
4. Wash plate (Section C, Step 3).
5. Secondary antibody, either streptavidin anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in blocking buffer. For a single 96 well plate, add 10 μ l of secondary antibody stock to 9.99 ml of blocking buffer. Mix well and add 100 μ l/well. Cover and incubate at 37°C for 30 min.
6. Wash plate (Section C, Step 3).
7. Add 100 μ l of TMB substrate per well. Cover and incubate at 37°C for 10 min.
8. Add 100 μ l of STOP solution per well. Shake gently for a few seconds.
9. Read plate on a microplate reader at absorbance 450 nm.
 1. **Visual Determination:** Read within 30 min after adding STOP solution.
 2. **Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP solution.

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