Store at 4°C

#7929

PathScan® Phospho-Syk (panTyr) Sandwich ELISA Antibody Pair

☑ 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

Introduction: CST's PathScan® Phospho-Syk (panTyr) Sandwich ELISA Antibody Pair is being offered as an economical alternative to our PathScan® Phospho-Syk (panTyr) Sandwich ELISA Kit #7928. Capture and detection antibodies (100X stocks) and HRP-conjugated secondary antibody (1000X stock) are supplied. Sufficient reagents are supplied for 4 x 96 well ELISAs. The Syk mouse capture antibody is coated in PBS overnight in a 96 well microplate. After blocking, cell lysates are added followed by biotinylated phospho-tyrosine mouse detection antibody and HRP-linked streptavidin. HRP substrate (TMB) is added for color development. The magnitude of the absorbance for this developed color is proportional to the quantity of Syk phosphorylated on tyrosines.

*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- PBS+0.05% Tween-20, 1% BSA

96 Well Microplates**

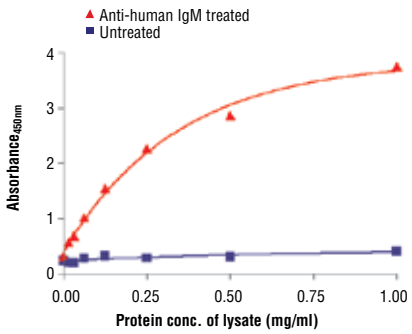
Microplate Reader

** Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592).

Note: Antibody pairs have been optimized using recommended buffers, reagents, plates and protocol. Solutions should be made fresh daily.

Background: Syk is a protein tyrosine kinase that plays an important role in intracellular signal transduction in hematopoietic cells (1-3). Syk interacts with immunoreceptor tyrosine-based activation motifs (ITAMs) located in the cytoplasmic domains of immune receptors (4). It couples the activated immunoreceptors to downstream signaling events that mediate diverse cellular responses, including proliferation, differentiation and phagocytosis (4). There is also evidence of a role for Syk in nonimmune cells, and Syk is a potential tumor suppressor in human breast carcinomas (5). Tyr323 is a negative regulatory phosphorylation site within the SH2-kinase linker region in Syk. Phosphorylation of Tyr323 provides a direct binding site to the TKB domain of Cbl (6,7). Tyrosine 352 of Syk is involved in the association of PLC-γ1 (8). Tyrosines 525 and 526 are located in the activation loop of the Syk kinase domain, and phosphorylation of Tyr525/526 of human Syk (equivalent to the Tyr519/520 of mouse Syk) is essential for Syk function (9).

Products Included	Item #	Volume	Cap Color	Storage
Syk Capture Ab (100X)	91011	400 µL	Pink	4°C
Phospho-Tyrosine Detection Mouse mAb (100X)	48478	400 µL	Blue	4°C
Anti-mouse IgG, HRP-linked Antibody (1000X)	16736	40 µL	Yellow	–20°C



The relationship between protein concentration of untreated or goat anti-human IgM treated Ramos cell lysates and the absorbance at 450 nm is shown. Cells were serum-starved overnight and then treated with goat anti-human IgM (10 µg/ml) for 10 min. at 37°C.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

(1) Cheng, A.M. and Chan, A.C. (1997) *Curr. Opin. Immunol.* 9, 528–533.

(2) Kurosaki, T. et al. (1997) *Curr. Opin. Immunol.* 9, 309–318.

(3) Chu, D.H. et al. (1998) *Immunol. Rev.* 165, 167–180.

(4) Turner, M. et al. (2000) *Immunol. Today* 21, 148–154.

(5) Coopman, P.J. et al. (2000) *Nature* 406, 742–747.

(6) Deckert, M. et al. (1998) *J. Biol. Chem.* 273, 8867–8874.

(7) Rao, N. et al. (2001) *EMBO J.* 20, 7085–7095.

(8) Law, C.L. et al. (1996) *Mol. Cell. Biol.* 16, 1305–1315.

(9) Zhang, J. et al. (2000) *J. Biol. Chem.* 275, 35442–35447.

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**—zebra fish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

PathScan® Sandwich ELISA Antibody Pair Protocol

A Required Reagents

1. **Coating Buffer:** 1X PBS, (20X PBS #9808)
   3.2 mM Na_2HPO_4, 0.5 mM KH_2PO_4, 1.3 mM KCl, 135 mM NaCl, pH 7.4
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)
   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, 1 µg/ml leupeptin.
5. **TMB Substrate:** (TMB Substrate #7004)
6. **STOP Solution:** (STOP Solution #7002)

NOTE: Reagents should be made fresh daily

B Coating Procedure

1. Rinse microplate with dH_2O. Add 200 µl of dH_2O and discard liquid. Blot on paper towel to make sure wells are dry.
2. Dilute capture antibody 1:100 in PBS. For a single 96 well plate, add 100 µl of Capture Antibody Stock to 9.9 ml PBS. Mix well and add 100 µl/well. Cover plate and incubate overnight at 4°C (17-20 hours).
3. **After overnight coating, gently uncover plate and wash wells:**
   a. Discard plate contents into a receptacle.
   b. Wash 4 times with 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.
   c. 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 hours.
5. After blocking, wash plate as in Step 3. Plate is ready to use.

C 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 diameter plate) 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.

D Test Procedure

1. Lysates can be used undiluted or diluted in Blocking Buffer. 100 µl of lysate is added per well. Cover plate and incubate at 37°C for 2 hours.
2. Wash plate as in Coating Procedure, Step 3.
3. Dilute detection antibody 1:100 in Blocking Buffer. For a single 96 well plate, add 100 µl of Detector Antibody Stock to 9.9 ml of Blocking Buffer. Mix well and add 100 µl/well. Cover plate and incubate at 37°C for 1 hour.
4. Plate is washed as in Coating Procedure, Step 3.
5. Secondary antibody, either, anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in Blocking Buffer. For a single 96 well plate, add 10 µl of secondary antibody stock to 9.99 ml of Blocking Buffer. Mix well and add 100 µl/well. Cover and incubate at 37°C for 30 minutes.
6. Wash plate as in Coating Procedure, Step 3.
7. Add 100 µl of TMB Substrate per well. Cover and incubate at 37°C for 10 minutes.
8. Add 100 µl of STOP Solution per well.
9. Read plate on a microplate reader at Absorbance 450 nm.