

# PathScan® Phospho-Src (Tyr416) Sandwich ELISA Antibody Pair



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✓ 1 Kit  
(4 X 96 assays)

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

## Species Cross-Reactivity: H, Dg

**Description:** Cell Signaling Technology's PathScan® Phospho-Src (Tyr416) Sandwich ELISA Antibody Pair is being offered as an economical alternative to our PathScan® Phospho-Src (Tyr416) Sandwich ELISA Kit #7953. 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-Src (Tyr416) rabbit capture antibody is coated onto a 96 well microplate overnight in PBS. After blocking, cell lysates are added followed by a total Src mouse detection antibody and anti-mouse IgG, HRP-linked antibody. HRP substrate (TMB) is then added for color development. The magnitude of the absorbance for this developed color is proportional to the quantity of phospho-Src (Tyr416).

## 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).

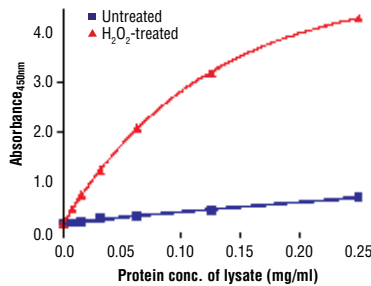
**Notes:** Antibody pairs have been optimized using recommended buffers, reagents, plates and the included protocol. Solutions should be made fresh daily.

**Background:** The Src family of protein tyrosine kinases, which includes Src, Lyn, Fyn, Yes, Lck, Blk and Hck, are important in the regulation of growth and differentiation of eukaryotic cells (1). Src activity is regulated by tyrosine phosphorylation at two sites, but with opposing effects. Phosphorylation of Src at Tyr416 in the activation loop of the kinase domain by carboxy-terminal Src kinase (Csk) upregulates enzyme activity, whereas phosphorylation of Tyr527 in the carboxy-terminal tail renders the enzyme less active (2).

## Background References:

- (1) Thomas, S.M. and Brugge, J.S. (1997) *Annu. Rev. Cell Dev. Biol.* 13, 513-609.
- (2) Hunter, T. (1987) *Cell* 49, 1-4.

Product Includes	Item #	Volume	Cap Color	Storage Temp
Phospho-Src (Tyr416) Capture Rabbit mAb (100X)	28293	400 µL	Pink	4°C
Src Detection Mouse mAb (100X)	41829	400 µL	Blue	4°C
Anti-mouse IgG, HRP-linked Antibody (1000X)	16736	40 µL	Yellow	-20°C



The relationship between the protein concentration of the lysate from untreated and H<sub>2</sub>O<sub>2</sub>-treated MDCK cells and the absorbance at 450 nm using the PathScan® Phospho-Src (Tyr416) Sandwich ELISA Antibody Pair #7963 is shown. MDCK cells were treated with H<sub>2</sub>O<sub>2</sub> (3 mM) for 3 minutes at 37°C and then lysed.

Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

## PathScan® Sandwich ELISA Antibody Pair Protocol

### A Required Reagents

- Coating Buffer:** 1X PBS, (20X PBS #9808)  
3.2 mM Na<sub>2</sub>HPO<sub>4</sub>, 0.5 mM KH<sub>2</sub>PO<sub>4</sub>, 1.3 mM KCl, 135 mM NaCl, pH 7.4
- Wash Buffer:** 1X PBS/0.05% Tween-20, (20X PBST #9809)
- Blocking Buffer:** 1X PBS/0.05% Tween-20, 1% BSA
- 1X Cell Lysis Buffer:**  
20 mM Tris-HCl (pH 7.5)  
150 mM NaCl  
1 mM disodium EDTA  
1 mM EGTA  
1% Triton  
20 mM sodium pyrophosphate  
25 mM sodium fluoride  
1 mM β-glycerophosphate  
1 mM Na<sub>3</sub>VO<sub>4</sub>  
1 μg/ml leupeptin
- TMB Substrate:** (TMB Substrate #7004)
- STOP Solution:** (STOP Solution #7002)

**NOTE:** Reagents should be made fresh daily

### B Coating Procedure

- Rinse microplate with dH<sub>2</sub>O. Add 200 μl of dH<sub>2</sub>O and discard liquid. Blot on paper towel to make sure wells are dry.
- 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).
- After overnight coating, gently uncover plate and wash wells:**
  - Discard plate contents into a receptacle.
  - 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.
  - Clean the underside of all wells with a lint-free tissue.
- Block plates. Add 150 μl of Blocking Buffer/well, cover plate and incubate at 37°C for 2 hours.
- After blocking, wash plate as in Step 3. Plate is ready to use.

### C Preparing Cell Lysates

- Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
- To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
- Remove PBS and add 0.5–1.0 ml ice-cold 1X PathScan® Sandwich ELISA Lysis Buffer plus 1 mM phenylmethylsulfonyl fluoride (PMSF) to each plate (10 cm in diameter) and incubate the plate on ice for 2 minutes.
- Collect cell lysate into new tubes. This cell lysate solution can be used directly in this ELISA Kit, or stored at –80°C in single-use aliquots.

### D Test Procedure

- 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.
- Wash plate as in Coating Procedure, Step 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.
- Plate is washed as in Coating Procedure, Step 3.
- 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.
- Wash plate as in Coating Procedure, Step 3.
- Add 100 μl of TMB Substrate per well. Cover and incubate at 37°C for 10 minutes.
- Add 100 μl of STOP Solution per well.
- Read plate on a microplate reader at Absorbance 450 nm.