

PathScan® Total Src Sandwich ELISA Kit



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✓ 1 Kit
(96 assays)

New 12/09

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Entrez-Gene ID #6714
Swiss-Prot Acc. #P12931

Species Cross-Reactivity: H, M

Description: The PathScan® Total Src Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of total Src protein. A Src rabbit antibody has been coated onto the microwells. After incubation with cell lysates, Src protein (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, a Src mouse detection antibody is added to detect the captured Src protein. Anti-mouse IgG, HRP-linked antibody is then used to recognize the bound detection antibody. The HRP substrate TMB is added to develop color. The magnitude of the absorbance for this developed color is proportional to the quantity of total Src protein.

Specificity/Sensitivity: The PathScan® Total Src Sandwich ELISA Kit #7984 from Cell Signaling Technology detects endogenous levels of total Src protein, as shown in Figure 1. This kit does not cross-react with the Src family members Yes, Lck, Lyn or Hck. Other family members have not been tested. The kit sensitivity is shown in Figure 2.

Background: The Src family of protein tyrosine kinases (including Src, Lyn, Fyn, Yes, Lck, Btk 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 Tyr416 in the activation loop of the kinase domain by 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.

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

Products Included	Volume	Solution Color
Src Rabbit Antibody Coated Microwells*	96 tests	
Src Mouse Detection Antibody	11 ml	green
Anti-mouse IgG, HRP-linked Antibody	11 ml	red
Sample Diluent	25 ml	blue
TMB Substrate	11 ml	colorless
STOP Solution	11 ml	colorless
Sealing Tape	2 sheets	
20X Wash Buffer	25 ml	colorless
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).

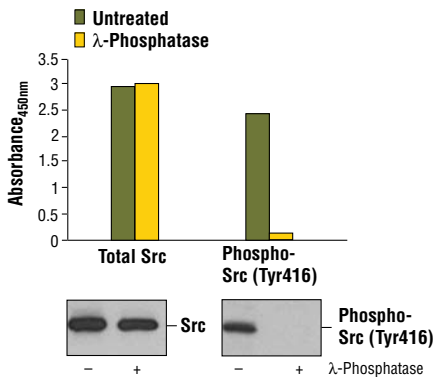


Figure 1. Constitutive phosphorylation of Src at Tyr416 in Calu-3 cells as detected by the PathScan® Phospho-Src (Tyr416) Sandwich ELISA Kit #7953. Treatment of lysate with λ-phosphatase significantly reduces Src phosphorylation but does not alter the levels of total Src protein as detected by the PathScan® Total Src Sandwich ELISA kit. Absorbance at 450 nm is shown in the top figure while the corresponding western blots using Src (L4A1) Mouse mAb #2110 (left) or Phospho-Src Family (Tyr416) (100F9) Rabbit mAb #2113 (right) are shown in the bottom figure.

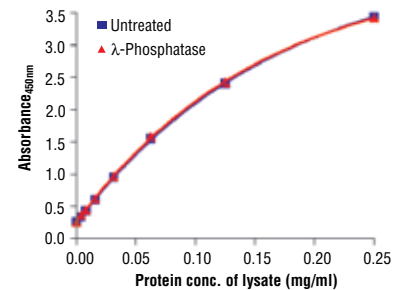


Figure 2. The relationship between lysate protein concentration from untreated and λ-phosphatase treated lysate from Calu-3 cells and the absorbance at 450 nm is shown.

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 Na₃VO₄, 1 μg/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 phenyl-methylsulfonyl 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.