

# PathScan® Phospho-c-Kit (panTyr) Sandwich ELISA Antibody Pair



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✓ 1 Kit  
(4 X 96 well plates)

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

## Species Cross-Reactivity: H

**Description:** Capture and Detection Antibodies (100X stocks) and HRP-Conjugated Streptavidin (1000X stock) are supplied. Sufficient reagents are supplied for 4 x 96 well ELISAs. The c-Kit 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-conjugated streptavidin. HRP substrate (TMB) is added for color development. The magnitude of the absorbance for this developed color is proportional to the quantity of phospho-c-Kit (panTyr) protein.

Antibodies in kit are custom formulations specific to 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).

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

**Specificity/Sensitivity:** For Antibody Pair specificity and sensitivity, please refer to the corresponding PathScan® Sandwich ELISA Kit. Note: This antibody pair detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

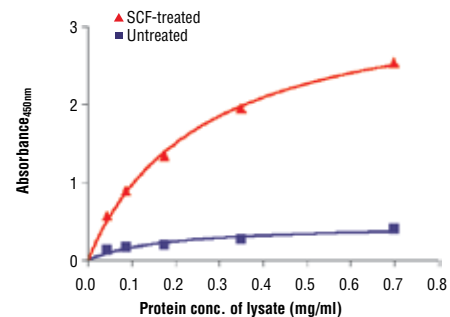
Product Includes	Item #	Volume	Cap Color	Storage
c-Kit Capture Mouse mAb (100X)	72087	400 µL	Pink	4°C
Biotinylated Phospho-Tyrosine Detection Ab (100X)	98264	400 µL	Blue	4°C
HRP-linked Streptavidin (1000X)	54986	40 µL	Clear	-20°C

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

**Background:** c-Kit is a member of the subfamily of receptor tyrosine kinases that includes PDGF, CSF-1, and FLT3/Flk-2 receptors (1,2). It plays a critical role in activation and growth in a number of cell types including hematopoietic stem cells, mast cells, melanocytes, and germ cells (3). Upon binding with its stem cell factor (SCF) ligand, c-Kit undergoes dimerization/oligomerization and autophosphorylation. Activation of c-Kit results in the recruitment and tyrosine phosphorylation of downstream SH2-containing signaling components including PLCγ, the p85 subunit of PI3 kinase, SHP2, and CrkL (4). Molecular lesions that impair the kinase activity of c-Kit are associated with a variety of developmental disorders (5), and mutations that constitutively activate c-Kit can lead to pathogenesis of mastocytosis and gastrointestinal stromal tumors (6). Tyr719 is located in the kinase insert region of the catalytic domain. c-Kit phosphorylated at Tyr719 binds to the p85 subunit of PI3 kinase *in vitro* and *in vivo* (7).

## Background References:

- (1) Martin, F.H. et al. (1990) *Cell* 63, 203-11.
- (2) Yarden, Y. et al. (1987) *EMBO J* 6, 3341-51.
- (3) Gommerman, J.L. et al. (1997) *J Biol Chem* 272, 30519-25.
- (4) Sattler, M. et al. (1997) *J Biol Chem* 272, 10248-53.
- (5) Nocka, K. et al. (1990) *EMBO J* 9, 1805-13.
- (6) Hirota, S. et al. (1998) *Science* 279, 577-80.
- (7) Blume-Jensen, P. et al. (2000) *Nat Genet* 24, 157-62.



The relationship between protein concentration of lysates from untreated and SCF-treated H526 lysates and the absorbance at 450 nm is shown. Cells ( $0.5 \times 10^6$  cells/ml) were serum starved overnight and then treated with Human Stem Cell Factor (hSCF) #8925 (100 ng/ml) for 5 min at 37°C, and then lysed.

## 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:** (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<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 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenyl-methylsulfonyl fluoride (PMSF) to each plate (10 cm<sup>2</sup>) and incubate the plate on ice for 5 minutes.
- Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
- Sonicate lysates on ice.
- 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

- 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.