

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



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✓ 1 Kit
(4 X 96 well plates)

New 01/08

This product is for *in vitro* research use only and is not intended for use in humans or animals.

Entrez-Gene ID # 16590
Swiss-Prot Acc. # P10721

Species Cross-Reactivity: H

Description: CST's PathScan® Phospho-c-Kit (panTyr) Sandwich ELISA Antibody Pair is being offered as an economical alternative to our PathScan® Phospho-c-Kit (panTyr) Sandwich ELISA Kit #7231. 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 this kit are custom formulations specific to the kit.

Reagents not supplied:

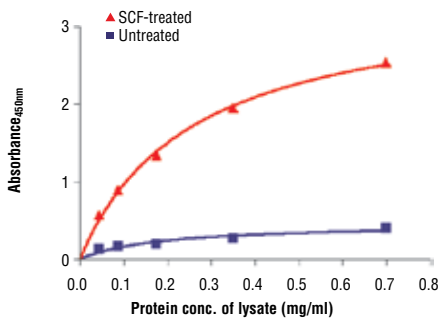
- Phosphate Buffered Saline (PBS-20X) #9808
- Phosphate Buffered Saline with Tween-20 (PBST-20X) #9809
- Cell Lysis Buffer (10X) #9803
- TMB Substrate #7004
- STOP Solution #7002
- Blocking Buffer: 1X PBS/0.05% Tween-20, 1% BSA
- 96 Well Microplates**
- Microplate Reader

** 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).

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

Background: c-Kit is a member of the subfamily of receptor tyrosine kinases that includes PDGF, CSF-1 and FLT3/Itk-2 receptors (1,2). It plays a critical role in activation and growth in a number of cell types such as hematopoietic stem cells, mast cells, melanocytes and germ cells (3). Upon binding with its ligand, stem cell factor (SCF), 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

Products Included	Volume	Cap Color	Storage
c-Kit Mouse Capture Antibody (100X)	0.4 ml	Pink	4°C
Biotinylated Phospho-Tyrosine Mouse Detection Antibody	0.4 ml	Blue	4°C
HRP-Linked Streptavidin (1000X)	0.04 ml	Natural	-20°C



The relationship between protein concentration of lysates from untreated and SCF-treated H526 lysates and the absorbance at 450 nm using PathScan® Phospho-c-Kit (panTyr) Sandwich ELISA Antibody Pair #7294 is shown. Cells (0.5x10⁶ cells/ml) were serum starved overnight and then treated with Human Stem Cell Factor (SCF) #9907 (40 ng/ml) for 5 min at 37°C.

variety of developmental disorders (5), while 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, 203–211.
- (2) Yarden, Y. et al. (1987) *EMBO J.* 6, 3341–3351.
- (3) Gommerman, J.L. et al. (1997) *J. Biol. Chem.* 272, 30519–30525.
- (4) Sattler, M. et al. (1997) *J. Biol. Chem.* 272, 10248–10253.
- (5) Nocka, K. et al. (1990) *EMBO J.* 9, 1805–1813.
- (6) Hirota, S. et al. (1998) *Science* 279, 577–580.
- (7) Blume-Jensen, P. et al. (2000) *Nat. Genet.* 24, 157–162.

Storage: c-Kit Mouse Capture Antibody and biotinylated Phospho-Tyrosine Mouse Detection Antibody are stored at 4°C.

HRP-Linked Streptavidin is stored at -20°C.

Companion Products:

- PathScan® Phospho c-Kit (panTyr) Sandwich ELISA Kit #7231
- PathScan® Total c-Kit Sandwich ELISA Kit #7197
- PathScan® Phospho-c-Kit (Tyr719) Sandwich ELISA Kit #7298
- Phospho-c-Kit (Tyr719) Antibody #3391
- c-Kit (Ab81) Mouse mAb #3308
- c-Kit Antibody #3392
- TMB Substrate #7004
- STOP Solution #7002
- Cell Lysis Buffer (10X) #9803
- Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411
- Phospho-Tyrosine Mouse mAb (P-Tyr-100) (Biotinylated) #9417
- Phosphate Buffered Saline (PBS-20X) #9808
- Phosphate Buffered Saline with Tween 20 (PBST-20X) #9809

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence F—Flow cytometry E—ELISA D—DELFIATM

Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish B—bovine 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

- Coating Buffer:** 1X PBS, (20X PBS #9808)
3.2 mM Na₂HPO₄, 0.5 mM KH₂PO₄, 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 Na₃VO₄, 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 dH₂O. Add 200 μl of dH₂O 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 cm²) 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.