Store at 4°C

#7231

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

Cell Signaling TECHNOLOGY®

☑ 1 Kit
(96 assays)

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New 11/07

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

Species Cross-Reactivity: H

Introduction: The PathScan® Phospho-c-Kit (panTyr) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of c-Kit when tyrosine phosphorylated. A c-Kit Mouse mAb* has been coated onto the microwells. After incubation with cell lysates, c-Kit (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, a Biotinylated Phospho-Tyrosine Mouse Detection Antibody* is added to detect tyrosine phosphorylation of the captured c-Kit protein. HRP-linked Strepavidin* is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of the absorbance for this developed color is proportional to the quantity of c-Kit phosphorylated on tyrosine.

* Antibodies in kit are custom formulations specific to kit.

Companion Products:

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

PathScan® Total c-Kit Sandwich ELISA Kit #7197

PathScan® Phospho-c-Kit (Tyr719) Sandwich ELISA Kit #7298

Specificity/Sensitivity: CST's PathScan® Phospho-c-Kit (panTyr) Sandwich ELISA Kit #7231 detects c-Kit when tyrosine phosphorylated. As shown in Figure 1, a significant induction of c-Kit tyrosine phosphorylation can be detected in H526 cells following treatment with SCF using the c-Kit (panTyr) Sandwich ELISA Kit #7231. The level of total c-Kit (phospho and nonphospho) remains unchanged as shown by Western analysis and by PathScan® Total c-Kit Sandwich ELISA Kit #7197. In Figure 3, Western blot analysis of protein captured in the c-Kit antibody coated microwell shows major band corresponding to the phospho-c-Kit protein.

Products Included	Volume	Solution Color
c-Kit Mouse mAb Coated Microwells*	96 tests	
Biotinylated Phospho-Tyrosine Mouse Detection Antibody	11 ml	green
HRP-Linked Streptavidin	11 ml	red
TMB Substrate	11 ml	colorless
STOP Solution	11 ml	colorless
Sealing Tape	2 sheets	
20X Wash Buffer	25 ml	colorless
Sample Diluent	25 ml	blue
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).

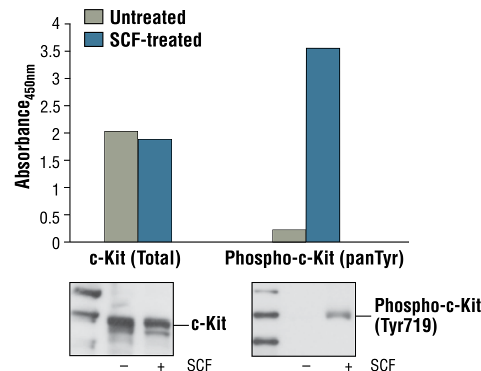

Figure 1. Treatment of H526 cells with SCF stimulates tyrosine phosphorylation of c-Kit, detected by PathScan® Phospho-c-Kit (panTyr) Sandwich ELISA Kit #7231, but does not affect the level of total c-Kit protein detected by PathScan® Total c-Kit Sandwich ELISA Kit #7197. The absorbance readings at 450 nm are shown in the top figure, while the corresponding Western blot using Phospho-c-Kit (Tyr719) Antibody #3391 (right panel) or c-Kit Antibody #3392 (left panel), is shown in the bottom figure.

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

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.

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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 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 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.

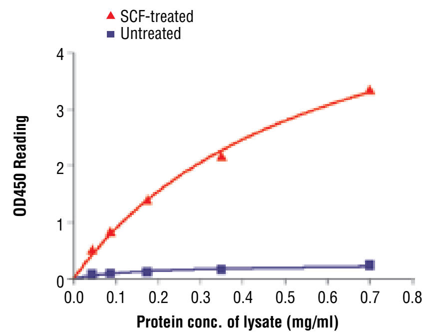

Figure 2. The relationship between protein concentration of lysates from untreated and SCF-treated H526 lysates and the absorbance at 450 nm is shown. Cells (0.5x10⁶ cells/ml) were serum starved overnight and then treated with SCF #9907 (40 ng/ml) for 5 min at 37°C.

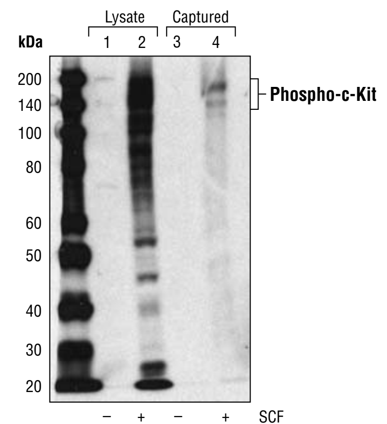

Figure 3. Kit specificity as demonstrated by Western analysis of the ELISA microwell captured protein. Lysates were prepared from untreated and SCF-treated H526 cells and incubated in microwells coated with the c-Kit capture antibody. Wells were washed and the captured protein was solubilized in SDS gel loading buffer. Western analysis of untreated and SCF-treated H526 cell starting lysates (lanes 1 & 2) and the captured protein (lanes 3 & 4) was carried out using Phospho-Tyrosine Mouse mAb (P-Tyr-100) (Biotinylated) #9417. The major band detected in the captured material corresponds to the phospho-c-Kit protein (lane 4).

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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 Na_3VO_4, 1µg/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 phenylmethylsulfonyl 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.

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