Revision 5

Store at 4°C

#7294

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

1 Kit (4 x 96 assays)

Species Cross Reactivity	UniProt ID:	Entrez-Gene Id:
H	#P10721	#3815

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Product Includes	Product #	Volume	Cap Color	Storage Temp
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 cellsignal.com for a complete listing of recommended companion products.

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/0.5% 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

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.

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken **Dm:** D. melanogaster
X: Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** horse
GP: Guinea Pig **Rab:** rabbit **All:** all species expected

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ELISA Antibody Pair

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS)**: (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **Wash Buffer**: 1X PBS/0.05% Tween® 20, (20X PBST #9809).
3. **Blocking Buffer**: 1X PBS/0.05% Tween® 20, 1% BSA.
4. **1X Cell Lysis Buffer**: 10X Cell Lysis Buffer (#9803): To prepare 10 ml of 1X Cell Lysis Buffer, add 1 ml of 10X Cell Lysis Buffer to 9 ml of dH_2O, mix. Buffer can be stored at 4°C for short-term use (1–2 weeks).

   Recommended: Add 1 mM phenylmethylsulfonyl fluoride (PMSF) (#8553) immediately before use.

5. **Bovine Serum Albumin (BSA)**: (#9998).
6. **TMB Substrate**: (#7004).
7. **STOP Solution**: (#7002)

   NOTE: Reagents should be made fresh daily.

B. Preparing Cell Lysates

For adherent cells

1. Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM PMSF to each plate (10 cm diameter) and incubate the plate on ice for 5 min.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 min (x14,000 rpm) 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.

For suspension cells

1. Remove media by low speed centrifugation (~1,200 rpm) when the culture reaches 0.5–1.0 x 10^6 viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1,200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
3. Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X cell lysis buffer plus 1 mM PMSF.
4. Sonicate lysates on ice.
5. Microcentrifuge for 10 min (x14,000 rpm) 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. Coating Procedure

1. Rinse microplate with 200 µl of dH_2O, discard liquid. Blot on paper towel to make sure wells are dry.
2. Dilute capture antibody 1:100 in 1X PBS. For a single 96 well plate, add 100 µl of capture antibody stock to 9.9 ml 1X PBS. Mix well and add 100 µl/well. Cover plate and incubate overnight at 4°C (17–20 hr).
3. **After overnight coating, gently uncover plate and wash wells**:
   1. Discard plate contents into a receptacle.
   2. Wash four times with wash buffer, 200 µl each time per well. For each wash, strike plates on fresh paper towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
   3. Clean the underside of all wells with a lint-free tissue.
4. Block plates. Add 150 µl of blocking buffer/well, cover plate, and incubate at 37°C for 2 hr.
5. After blocking, wash plate (Section C, Step 3). Plate is ready to use.

D. Test Procedure

1. 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 hr.
2. Wash plate (Section C, Step 3).
3. Dilute detection antibody 1:100 in blocking buffer. For a single 96 well plate, add 100 µl of detection 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 hr.
4. Wash plate (Section C, Step 3).
5. Secondary antibody, either streptavidin 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

min.
6. Wash plate (Section C, Step 3).
7. Add 100 µl of TMB substrate per well. Cover and incubate at 37°C for 10 min.
8. Add 100 µl of STOP solution per well. Shake gently for a few seconds.
9. Read plate on a microplate reader at absorbance 450 nm.
    1. **Visual Determination**: Read within 30 min after adding STOP solution.
    2. **Spectrophotometric Determination**: Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP solution.

posted January 2008

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