

## ProductInformation

### Anti-phospho-c-Kit (SCFR) [pTyr<sup>936</sup>]

Developed in Rabbit, Affinity Isolated Antibody

Product Number **C6365**

#### Product Description

Anti-phospho-c-Kit (SCFR) [pTyr<sup>936</sup>] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of human c-Kit that contains tyrosine 936 as immunogen. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated c-Kit.

The antibody detects human c-Kit. Cow (100% homologous), dog (92% homologous), and mouse and rat (85%), c-Kit have not been tested, but are expected to react. Chicken (23%) is not expected to react. The antibody has been used in immunoblotting applications.

c-Kit, also known as CD117 and stem cell factor receptor (SCFR), is a 145 kDa transmembrane tyrosine kinase encoded by the c-Kit proto-oncogene. c-Kit acts to regulate a variety of biological responses including cell proliferation, apoptosis, chemotaxis and adhesion. Ligand binding to the extracellular domain leads to autophosphorylation on several tyrosine residues within the cytoplasmic domain, and activation. c-Kit mutations correlate with tumor growth and progression in a variety of cancers including mast cell disease, gastrointestinal stromal tumor, acute myeloid leukemia, Ewing sarcoma, and lung cancer.

Phosphorylation at tyrosine 936 of c-Kit allows binding of Grb2 and activation of the Ras → Raf → ERK1&2 signaling pathway.

#### Reagent

The antibody is supplied as 100 µL in Dulbecco's phosphate buffered saline (without Mg<sup>2+</sup> and Ca<sup>2+</sup>), pH 7.3, with 50% glycerol, 1.0 mg/ml BSA (IgG and protease free) and 0.05% sodium azide

#### Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

#### Storage/Stability

Store at -20 °C. Due to the presence of 50% glycerol the antibody will remain in solution. For extended storage, centrifuge the vial briefly before opening and prepare working aliquots. To ensure accurate dilutions mix gently, remove excess solution from pipette tip with clean absorbent paper, pipette slowly. The antibody is stable for at least six months when stored appropriately. Working dilutions should be discarded if not used within 12 hours.

#### Product Profile

The supplied reagent is sufficient for 10 blots.

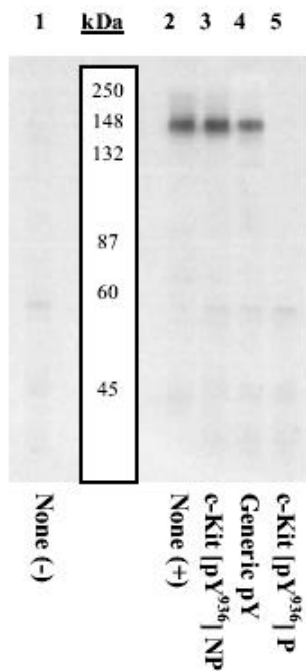
A recommended working dilution 1:1000 is determined by immunoblotting using M07e cells +/- SCF.

**Note:** In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

#### Peptide Competition

1. Extracts prepared from M07e cells were left untreated (lane 1) or treated (lanes 2-5) with SCF.
2. Extracts were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF.
3. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C.
4. The membranes were preincubated as follows:  
Lane 1 & 2 no peptide  
Lane 3 the non-phosphopeptide corresponding to the immunogen  
Lane 4 a generic phosphotyrosine containing peptide  
Lane 5 the phosphopeptide immunogen
5. Following preincubation, all membranes were incubated with 0.50 µg/mL c-Kit [pTyr<sup>936</sup>] antibody for two hours at room temperature in a 1% BSA-TBST buffer.
6. After washing, membranes were incubated with goat F(ab')<sub>2</sub> anti-rabbit IgG alkaline phosphatase and signals were detected using the Tropix WesternStar<sup>™</sup> method.

The data show that only the peptide corresponding to c-Kit [pTyr<sup>936</sup>] blocks the antibody signal, thereby demonstrating the specificity of the antibody.



## References

1. Liang, X., et al., Phosphatidylinositol 3-kinase and Src family kinases are required for phosphorylation and membrane recruitment of Dok-1 in c-Kit signaling., *J. Biol. Chem.*, **277**, 13732-13738 (2002).
2. Taylor, M.L., and Metcalfe, D.D., Kit signal transduction., *Hematol. Oncol. Clin. North Am.*, **14**, 517-535 (2000).
3. Demetri, G.D., Targeting c-kit mutations in solid tumors: scientific rationale and novel therapeutic options., *Semin. Oncol.* **28**, 19-26 (2001).
4. Thommes, K., et al., Identification of Tyr-703 and Tyr-936 as the primary association sites for Grb2 and Grb7 in the c-Kit/stem cell factor receptor. *Biochem. J.*, **341**, 211-216 (1999).

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