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ProductInformation

Anti-phospho-c-Kit (SCFR) (pTyr^{730})
produced in rabbit, affinity isolated antibody

Catalog Number **C6615**

Product Description

Anti-phospho-c-Kit (SCFR) (pTyr^{730}) is produced in rabbit using as immunogen a synthetic phosphorylated peptide derived from the region of human c-Kit (Gene ID: 3815) that contains Tyr^{730}. 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. Mouse, rat and cow (100% homologous), chicken (85%), or dog (92%) c-Kit have not been tested, but are 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 Tyr^{730} of c-Kit allows binding of PLCγ and signaling to multiple pathways.

Reagent

The antibody is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3, with 1.0 mg/ml BSA (IgG and protease free) and 0.05% sodium azide.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at –20 °C. Upon initial thawing freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. Do not store in frost-free freezers. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 12 months when stored appropriately.

Product Profile

The supplied reagent is sufficient for 10 blots.

Immunoblotting: a recommended working concentration of 0.1-1.0 µg/mL is determined 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^{730}) antibody for two hours at room temperature in a 1% BSA-TBST buffer.
6. After washing, membranes were incubated with goat F(ab')$_2$ anti-rabbit IgG alkaline phosphatase and signals were detected.

The data show that only the peptide corresponding to c-Kit (pTyr^{730}) blocks the antibody signal, thereby demonstrating the specificity of the antibody. (Figure 1)

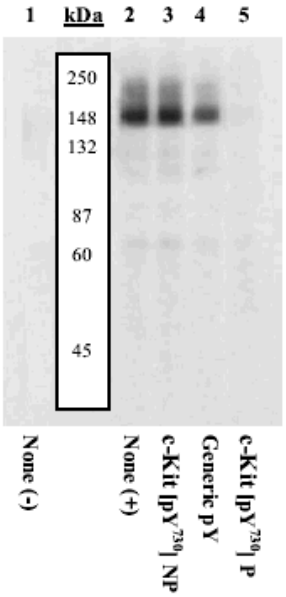

Figure 1 – Peptide competition

References

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2. Taylor, M.L., and D.D. Metcalfe Kit signal transduction. *Hematol. Oncol. Clin. North Am*., **14**, 517-535 (2000).
3. Gommerman, J.L., et al., Differential stimulation of c-Kit mutants by membrane-bound and soluble Steel Factor correlates with leukemic potential. *Blood*, **96**, 3734-3742 (2000).

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