3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Anti-phospho-c-Kit (SCFR) (pTyr^{703})
Developed in Rabbit, Affinity Isolated Antibody

Product Number **C 6740**

Product Description

Anti-phospho-c-Kit (SCFR) (pTyr^{703}) is developed in rabbit using a synthetic phosphorylated peptide derived from the region of human c-Kit that contains tyrosine 703 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. Mouse, rat, cow and dog (92% homologous), or chicken (69%) c-Kit have not been tested, but are expected to react. It has been used in immunoblotting applications.[1]

c-Kit, also known as CD117 and stem cell factor receptor, 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 703 of c-Kit allows binding of Grb2 and activation of the Ras → Raf → ERK1&2 signaling pathway.

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

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 –70 °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.

A recommended working concentration of 0.1 to 1.0 µg/mL 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:
   Lanes 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^{703}] 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^{703}] blocks the antibody signal, thereby demonstrating the specificity of the antibody. (Figure 1)

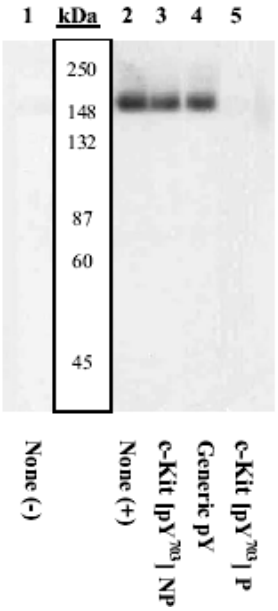

Figure 1 – Peptide competition

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. 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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