



Product Information

Anti-phospho-c-Raf (pSer⁶²¹)

Developed in Rabbit, Affinity Isolated Antibody

Product Number **R 1151**

Product Description

Anti-phospho-c-Raf (pSer⁶²¹) was developed in rabbit using a synthetic phosphopeptide derived from a region of human c-Raf that contains serine 621 as immunogen. The serum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity with non-phosphorylated c-Raf.

Anti-phospho-c-Raf (pSer⁶²¹) recognizes the phosphorylated form of human c-Raf that contains a phosphate on serine 621 by immunoblotting. Mouse and rat c-Raf (100% homologous) have not been tested but are expected to react.

c-Raf, also known as Raf-1 is a member of the MEK kinase family. The major phosphorylation sites of Raf-1 kinase are serine 621 and tyrosines 340/341.^{1,2,3} Phosphorylation of serine at position 621 is crucial to catalytic activity and down-regulation by cAMP.^{5, 6} This serine/threonine kinase is activated by IL-3 receptor, EGF, PMA, and other growth factors. Activation of c-Raf by GTP-bound Ras causes phosphorylation of MEK, which leads to activation of MAPK/ERK kinases.⁴

Reagent

The antibody is supplied as 100 μ L of a 0.50 mg/mL solution in Dulbecco's phosphate buffered saline (without Mg²⁺ and Ca²⁺), pH 7.3, with 1.0 mg/mL BSA (IgG, protease free) as a carrier 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.

Storage/Stability

Store at -70°C . For extended storage, upon initial thawing, freeze in working aliquots. Avoid repeated freezing and thawing to prevent denaturing the antibody. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 6 months when stored appropriately.

Product Profile

The supplied reagent is sufficient for 10 immunoblots.

A recommended working concentration of 0.1 to 1.0 $\mu\text{g/ml}$ is determined for immunoblotting using immunoprecipitates or cell lysates from EGF-stimulated Hek293 cells transfected with c-Raf.

Peptide competition data demonstrates that only the phosphopeptide corresponding to the region containing serine 621 blocks the antibody signal, which confirms the specificity of the Anti-phospho-c-Raf [pSer⁶²¹] for this phosphorylated residue.

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

References

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7. Thorson, J.A., et al., 14-3-3 proteins are required for maintenance of Raf-1 phosphorylation and kinase activity., *Mol. Cell. Biol.*, **18**, 5229-5238 (1998).

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