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ProductInformation

Anti-phospho-c-Raf (pTyr $^{340/341}$)
Developed in Rabbit, Affinity Isolated Antibody

Product Number **R 1026**

Product Description

Anti-phospho-c-Raf (pTyr$^{340/341}$) was developed in rabbit using a synthetic phosphopeptide derived from a region of human c-Raf that contains tyrosines 340 and 341 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 (pTyr$^{340/341}$) recognizes the phosphorylated form of human c-Raf that contains a phosphate on tyrosines 340 and 341. 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 serines: 43, 259 and 621 and tyrosines 340, 341.[1,2,3] Phosphorylation of c-Raf at these sites plays a major role in Raf activation and regulation. The phosphorylation of c-RAF occurs in response to IL-3 receptor, EGF, PMA, IGF-I, and other growth factors and is crucial for the Raf-1 activation. Activation of Raf by GTP-bound Ras causes phosphorylation of MEK, which leads to the activation of MAPK/ERK kinases. Phosphorylation of tyrosines at position 340 and 341 induces the ability of the protein kinase to phosphorylate MEK.[4] The Raf kinases play an important and specific role in the activation of extracellular signal-regulated kinases (ERK) cascade. Raf-1 is involved in regulation of proliferation, differentiation and apoptosis. ATP inhibits growth factor-induced activation of c-Raf-1.[6]

Reagent

The antibody is supplied as 100 µL of 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), 50% glycerol 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 –20 °C. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 6 months if stored appropriately.

Product Profile

The supplied reagent is sufficient for 10 immunoblots.

A recommended working dilution of 1:1000 is determined by immunoblotting using HEK293 cells overexpressing c-Raf, *in vitro*-phosphorylated by active Src.

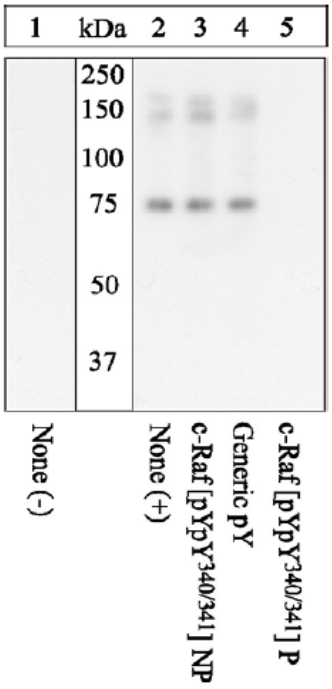
Peptide Competition

1. Lysates prepared from HEK293 cells overexpressing c-Raf were left unstimulated (Lane 1) or stimulated with active Src (Lanes 2-5), resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
2. Membranes were blocked with a 5% BSA-TBST buffer for 1 hour at room temperature.
3. After blocking, membranes were preincubated with different peptides as follow:

Lanes 1, 2	no peptide
Lane 3	non phosphorylated peptide corresponding to the immunogen
Lane 4	a generic phosphotyrosine containing peptide
Lane 5	immunogen

4. After preincubation membranes were incubated with Anti-phospho-c-Raf ($pTyr^{340/341}$) for two hours at room temperature in a 1% BSA-TBST buffer.
5. 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-Raf ($pTyr^{340/341}$) blocks the antibody signal, thereby demonstrating the specificity of the antibody 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

1. Morrison, D.K., et al., Identification of the major phosphorylation sites of the Raf-1 kinase., J. Biol. Chem., **268,** 17309-17316 (1993).
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3. Diaz, B., et al., Phosphorylation of Raf-1 serine 338-serine 339 is an essential regulatory event for Ras-dependent activation and biological signaling., Mol. Cell. Biol., **17,** 4509-4516 (1997).
4. Jelinek, T., et al., Ras-induced activation of Raf-1 is dependent on tyrosine phosphorylation., Mol. Cell. Biol., **16,** 1027-1034 (1996).
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6. Lenz, G., et al., Extracellular ATP stimulates an inhibitory pathway towards growth factor-induced cRaf-1 and MEKK activation in astrocyte cultures., J. Neurochem., **77,** 1001-1009 (2001).

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