

## Product Information

## Anti-Raf-1/c-Raf antibody, Mouse monoclonal

clone RNP1, purified from hybridoma cell culture

**R2404**

### Product Description

Monoclonal Anti-Raf-1/c-Raf (mouse IgG1 isotype) is derived from the RNP1 hybridoma produced by the fusion of mouse myeloma cells (NS1) and splenocytes from BALB/c mice immunized with a synthetic peptide corresponding to amino acids 334-345 of human Raf-1. The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2.

Monoclonal Anti-Raf-1/c-Raf recognizes human, rat and mouse Raf-1 (~75 kDa). The antibody may also detect Raf-1 degradation products as additional weaker bands at ~50 kDa. The product is useful in ELISA, immunoblotting, immunoprecipitation and immunocytochemistry.

Raf-1 (c-Raf-1), a cytoplasmic serine/threonine protein kinase (73-76 kDa). It belongs to a small family of protein kinases that have been identified as central in signal transduction pathways.<sup>1-3</sup> Raf-1 is the prototype member of this family that includes Raf-A and Raf-B, and is the cellular homolog of the viral oncogene v-Raf. Raf-1 is widely expressed in many tissues, whereas Raf-A and Raf-B are expressed mainly in urogenital and brain tissues, respectively<sup>4</sup> Raf-1 functions in the control of cell growth and differentiation, and its oncogenic form can initiate malignant transformation of cells. Raf-1 has a critical role in the Ras/MAP kinase signaling pathway, integrating mitogenic signals in mammalian cells from many growth factors, cytokines and oncogenes, e.g., EGF, PDGF, insulin, IL-2, IL-3, CSF-1, and GM-CSF. Raf-1 contains three regions that are highly conserved among Raf proteins, namely CR1, CR2, and CR3. CR1 is rich in cysteine residues and contains two Ras binding domains, CRD and RBD. CR2 is rich in serine/threonine residues and CR3 is located within the C-terminal region of Raf-1 and constitutes the protein kinase domain. The regulation of Raf-1 activity is a highly complex, multistep process whose regulatory events are not fully understood. Inactive Raf-1 is found in the cytosol, and is constitutively associated with the chaperones Hsp90, p50, and the 14-3-3 protein.<sup>5, 6</sup>

Upon mitogenic stimulation, Raf-1 directly interacts with Ras-GTP.<sup>7</sup> This interaction with activated Ras localizes Raf-1 to the plasma membrane and is the first step in its activation. Raf-1 activity is regulated by autophosphorylation at threonine residues and by phosphorylation at multiple serine and tyrosine residues by a number of protein kinases, including PKC, Src and JAK2. Raf-1 regulates the MAP kinase pathway by phosphorylating and activating the downstream MAP kinase kinase, MEK.<sup>8-10</sup>

### Reagent

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

Antibody concentration: ~2.0 mg/mL

## Precautions and Disclaimer

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

## Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

## Product Profile

Immunoblotting: a working antibody concentration of 2.0-4.0 µg/mL is determined using extracts of A431 cell line (human epidermoid carcinoma).

**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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