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ProductInformation

Anti-Raf-1
Developed in Rabbit
IgG Fraction of Antiserum

Product Number: **R 5773**

Product Description

Anti-Raf-1 is developed in rabbits using a synthetic peptide K-TEDINACTLTTSPRLPVF corresponding to the C-terminal of Raf-1 of human origin (amino acids 631-648 with N-terminally added lysine), conjugated to KLH as immunogen. This sequence is identical in rat Raf-1 and v-Raf from mouse sarcoma virus. Whole antiserum is fractionated and then further purified by ion-exchange chromatography to provide the IgG fraction of antiserum that is essentially free of other rabbit serum proteins.

Anti-Raf-1 recognizes Raf-1 (74 kDa) by immunoblotting and immunoprecipitation. The antibody may also detect Raf-1 degradation products as additional weaker bands at 40-60 kD. Staining of the Raf-1 in immunoblotting is specifically inhibited with Raf-1 immunizing peptide (Raf-1 human, amino acids 631-648 with N-terminally added lysine).

Raf-1 (c-Raf-1), a cytoplasmic serine/threonine protein kinase (73-76kD), belongs to a small family of protein kinases that have been identified as central in signal transduction pathways.[1-3] Raf-1 is the prototype member of this family that include 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 the urogenital and brain tissues, respectively.[4] Raf-1 functions in the control of cell growth, 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 mammalian cells from many growth factors, cytokines and oncogenes including 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, 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.[5, 6] Upon mitogenic stimulation of cells, Raf-1 directly interacts with Ras-GTP.[7] 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.[8]

Reagents

The product is provided as IgG fraction of antiserum in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM 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 hazardous 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 is not recommended. 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

By immunoblotting, a minimum working dilution of 1:8,000 is recommended using a lysate of the human epidermal carcinoma A431 cell line.

The antibody may be used in immunoprecipitation of Raf-1 using 5 µg IgG with Protein A-agarose and 10 µg lysate of cultured A431 cells.

Note: In order to obtain the best results and assay sensitivity in various techniques and preparation,s we recommend determining optimal working dilutions by titration.

References

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