

Product Information

B-Raf, ACTIVE

Human, Recombinant
Expressed in Sf9 Insect Cells

Product Number **B 1307**

Product Description

B-Raf, Active is produced from DNA sequence encoding full length human B-Raf with C-terminal His-tag expressed Sf9 insect cells. The recombinant protein appears as a band of 92 kDa on SDS-PAGE. Three other bands of 23, 32, and 66 kDa are also apparent. B-Raf, Active is suitable for phosphorylation assays.

B-Raf is a proto-oncogene that is a member of the serine/threonine protein kinase family. Mammals possess three Raf proteins: Raf-1, A-Raf and B-Raf. All are involved in signal transduction from the membrane to the nucleus.^{1,2} They play important roles in cellular processes such as proliferation, differentiation and apoptosis.

Studies of knock-out mice indicate that the three isoforms of Raf are not redundant, but rather serve different functions.³ B-Raf is highly expressed in brain tissues and is also found in testes.⁴ It appears to play a role in neural differentiation and in the maturation and maintenance of endothelial cells. A-Raf, found predominantly in urogenital tissues, plays a role in intestinal and neurological development, whereas Raf-1 appears to play a more general role in tissue formation.

B-Raf is activated by Ras.^{5,6} Once active, this serine/threonine kinase then activates MEK1 (Mitogen Activated Protein Kinase Kinase 1) which in turn activates Erk (Extra-cellular-signal-regulated kinase).^{7,8} High levels of cAMP have been reported to inhibit both B-Raf and Raf-1.⁷⁻⁹ However, elevating cAMP in PC12 cells maintained in serum-containing medium, inhibited B-Raf but not Raf-1, suggesting that B-Raf and Raf-1 are differentially regulated by cAMP-dependent kinases during cell proliferation. It has also been shown that B-Raf can be activated by the small G protein Rap1 both *in vitro* and *in vivo* via a Ras-independent pathway.^{10,11}

The Ras/Raf signaling pathway is crucial for cell proliferation. The corruption of this pathway can result in the initiation and/or progression of human cancers.

Thus, a thorough understanding of this pathway will be crucial in delineating treatment for cancers.

Reagent

Each vial contains 10 units active B-Raf in 50 mM Tris-HCl, pH 7.5, 0.27 M sucrose, 1 mM EDTA, 1 mM EGTA, 1 mM sodium orthovanadate, 10 mM β -glycerophosphate, 50 mM NaF, 5 mM sodium pyrophosphate, 1 % Triton X-100, 0.1 % 2-mercaptoethanol, 0.2 mM PMSF, 1 mM benzamide.

Storage/Stability

Store at -70°C . Centrifuge original vial after thawing and prior to removing the cap for maximum recovery of product. Avoid repeated thawing and freezing. Do not store in a frost-free freezer.

Product Profile

B-Raf activity is measured by its ability to activate MAP kinase 2 (Erk2) in the presence of MEK1. Once activated, MAP kinase 2 then phosphorylates myelin basic protein. One unit of B-Raf activity is then defined as that amount which results in 1 nmole of phosphate incorporated into myelin basic protein per minute at pH 7.2 at 30°C .

References

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