ProductInformation

Raf-1 (Δ 1-306), ACTIVE
Human, Recombinant
Expressed in Sf9 Insect Cells

Product Number **R 9276**

Product Description

Raf-1 (Δ 1-306), Active is produced from DNA sequence encoding human Raf-1, with N-terminal GST-tag, lacking amino acids 1-306. It is expressed in Sf9 cells and purified by GSH-agarose. The apparent MW of Raf-1 (Δ 1-306) is approximately 62 kDa. Bands at 90 kDa (HSP90), 34-36 kDa (14-3-3 proteins) and 26-28 kDa (Sf9 GSH-agarose binding proteins) are also detected by SDS-PAGE. Raf-1 (Δ 1-306) is suitable for use in kinase assays.

Raf-1 is a proto-oncogene product and a member of the serine/threonine protein kinase family. It participates in protein kinase cascades involved in mitogenic signaling. Raf-1 is uniformly and ubiquitously expressed in the cytoplasm. Raf-1 is highly conserved from Drosophila to mammals. It has been shown to be both an effector of the Ras oncoprotein as well as an activator of the MAP kinase pathway.

Raf-1 activation *in vivo* occurs at the plasma membrane and requires that Raf-1 bind the effector loop of the GTP bound form of Ras. This interaction results in recruitment of Raf-1 to the plasma membrane where it is then activated.[1,2] The Raf-Ras interaction can be bypassed if Raf-1 is constitutively localized to the plasma membrane.[1,2] However, Ras has been shown to interact with two N-terminal regions of Raf-1 (RID/RBS1 and Raf-CRD[3,4]) suggesting that Ras may also be involved in Raf-1 activation as well as recruitment to the plasma membrane.

Raf-1 activation involves other components such as the 14-3-3 family of proteins. The interaction of Raf-1 with 14-3-3 proteins protects active Raf-1 from phosphatase action.[5] Important regulatory phosphorylation events affecting Raf activity occur on tyrosines 340 and 341 and serines 259 and 499 (activating), serine 43 (prevents Ras:GTP binding), and serine 621 (constitutive, required for activity).[6]

Once active, Raf-1 phosphorylates and activates MAP kinase kinase (MEK) which, in turn, activates MAP kinase (ERK). ERK then phosphorylates and activates cytoplasmic targets such as Rsk[7] and Mnk[8,9] and/or translocates to the nucleus where it stimulates the activity of various transcription factors such as Elk-1. Activation of Elk-1 results in changes in gene expression.

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 GST-Raf-1(Δ 1-306) in 50 mM Tris-HCl, pH 7.5, 0.27 M sucrose, 0.15 M NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35.

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 frost-free freezer.

Product Profile

Raf-1 (Δ 1-306) activity is measured by its ability to activate MEK1 resulting in the subsequent activation of MAP kinase 2(Erk2). Once activated, MAP kinase 2 phosphorylates myelin basic protein. One unit of Raf-1 (Δ 1-306) activity is defined as the amount which results in 1 nmole of phosphate incorporated into myelin basic protein per minute at pH 7.2 at 30 °C.

Purity: Approximately 30%.

References

1. Leevers, S.J., et al., Nature, **369**, 411, (1994).
2. Stokoe, D., et al., Science, **264**, 1463 (1994).
3. Fabian, J.R.,et al., Proc. Natl. Acad. Sci. USA, **91**, 5982 (1994).
4. Brtva, T.R., et al., J. Biol. Chem., **270**, 9809 (1995).
5. Dent, P., et al., Science, **268**, 1902 (1995).
6. Daum, G., et al., Trends Biochem. Sci., **19**, 474 (1994).

7. Sturgill, T.W., et al., Nature, **334**, 715 (1988).
8. Waskiewicz, A.J., et al., EMBO J, **16**, 1090 (1997).
9. Wang, X., et al., J. Biol. Chem., **273**, 9373 (1998).

LLK 8/01