ProductInformation

ANTI-RAF-1 (253-269)
Developed in Rabbit, IgG Fraction of Antiserum

Product Number **R7648**

Product Description

Anti-Raf-1 is developed in rabbit using a synthetic peptide (QRQRSTSTPNVHMVSTT) that corresponds to human Raf-1 (amino acids 253-269) as immunogen. This sequence is the conserved proline, serine, threonine rich domain that lies before the catalytic domain. The antibody is purified using protein A chromatography.

Anti-Raf-1 specifically reacts with human Raf-1 (74 kD) and Raf-A (68 kD). The immunizing sequence is 100% conserved in human, rat, chicken and frog and shows 94% homology to A-Raf and 53% homology to human B-Raf. The antibody cross-reacts with mouse, rat, chicken and *Xenopus*.

Anti-Raf-1 may be used for immunoprecipitation and immunoblotting of Raf-1and Raf-A.

Raf-1 and Raf-A are serine/threonine protein kinases. Raf-1 is uniformly and ubiquitously expressed in the cytoplasm while Raf-A is more variably expressed. Raf-A is highly expressed in urogenital and kidney tissues and, to a lesser extent, brain tissue. Raf-1 is highly conserved from *Drosophila* to mammals.

Raf-1 has been shown to be both an effector of the Ras oncoprotein as well as an activator of the MAP kinase pathway. Specifically, Raf-1 binds the effector loop of Ras when it complexes with GTP. 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 involved in Ras activation 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. Antibodies to Raf-1 may be used to study their expression and function in a variety of cell types and tissues. Moreover, their expression pattern can be correlated with physiological functions or pathological conditions.

Reagents

The product is supplied as IgG fraction in 0.07 M Tris-glycine buffer, pH 7.4, containing 0.105M NaCl, 30% glycerol and 0.035% sodium azide (see MSDS)* as a preservative.

Protein concentration is approximately 1 mg/ml by Bradford analysis.

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

Store at 0°C to –20°C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1µg/µl total cell protein in a microcentrifuge tube with PBS (Sigma Product No. P3813).
2. Add 4 µg of anti-Raf-1 to 500 µg - 1mg cell lysate.

3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 µl of a washed (in PBS) 1:1 slurry of Protein A-Agarose beads (50 µl packed beads) (Sigma Product No. P2545).
5. Gently rock reaction mixture at 4°C for 2 hours.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice cold cell lysis buffer or PBS.
7. Resuspend the agarose beads in 50 µl 2X Laemmli sample buffer. The agarose beads can be frozen for later use.
8. Suspend the agarose beads in Laemmli sample buffer and boil for 5 minutes. The beads are pelleted by a microcentrifuge pulse. SDS-PAGE and subsequent immunoblotting analysis may be performed on a sample of the supernatant.

Lysis Buffer:
50 mM Tris-HCl, pH 7.4, containing 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1 mM PMSF, 1 µg/ml each aprotinin, leupeptin, pepstatin, 1 mM Na_3VO_4, and 1 mM NaF.

Product Profile
Recommended use: 4 µg of Anti-Raf-1 will immunoprecipitate Raf-1 from 0.5-1 mg of a cell lysate of human A431 jurkat cells. Recommended working concentration is 1-2 ug/ml of Anti-Raf-1 by immunoblotting using human A431 jurkat and mouse 3T3 fibroblast cell lysates and enhanced chemiluminescence.

Note: In order to obtain best results and assay sensitivity in different techniques and preparations we recommend determining optimal working dilutions by titration test.

References

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