ProductInformation

ANTI-RAF-1 (637-648)
Developed in Sheep, Affinity Isolated Antibody

Product Number **R7773**

Product Description

Anti-Raf-1 is developed in sheep using a synthetic peptide (CTLTTSPRLPVF) that corresponds to the C-terminal of human Raf-1 (amino acids 637-648) as immunogen. Affinity isolated antibody is obtained by immunospecific purification using the immunizing peptide.

Anti-Raf-1 specifically reacts with human Raf-1 (74 kD) and does not cross-react with A-Raf or B-Raf. The antibody cross-reacts with mouse and rat Raf-1. The antibody does not interfere with kinase activity. By immunoprecipitation, the antibody immunoprecipitates Raf-1 from recombinant human Raf-1 expressed in Sf9 insect cells with Ras and Lck. The immunocomplex was then used in a coupled phosphorylation assay to activate Mek1 that activated the MAPK-2, which phosphorylated the MBP substrate *in vitro*.

Anti-Raf-1 may be used for immunoprecipitation of Raf-1 but is not recommended for immunoblotting.

Raf-1 is a cytoplasmic serine/threonine protein kinase that 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 the affinity isolated antibody in 0.07 M Tris-glycine buffer, pH 7.4, containing 30% glycerol and 0.035% sodium azide (see MSDS)* as a preservative.

Protein concentration is approximately 0.7 mg/ml by Bradford.

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. Add 5 µg of Anti-Raf-1 to a microcentrifuge tube.
2. Add 100 µl of a 1:1 (v/v) slurry of Protein G-agarose (Sigma Product No. P2294) that has been washed in PBS.
3. Incubate for 30 minutes to 1 hour at 4°C in PBS.
4. Pellet at 14,000 rpm for 15 seconds .

5. Remove the supernatant and then wash the antibody/protein G-agarose twice with Buffer A (50 mM Tris, pH 7.5, 1 mM EDTA, 1 mM EGTA, 0.5 mM Na_3VO_4, 0.1 % 2-mercaptoethanol, 1% Triton X-100, 50 mM NaF, 5 mM sodium pyrophosphate, 10 mM sodium glycerophosphate, 0.1 mM PMSF, 1 µg/ml of aprotinin and leupeptin).
6. Resuspend the pellet of washed beads in 100 µl of Buffer A.
7. Add sample containing antigen to the beads.[a]
8. Incubate for 2 hours at 4°C.
9. Wash the antigen/antibody/protein G-agarose complex with 500 µl of Buffer A containing 0.5 M NaCl by centrifuging in a microcentrifuge for 5 seconds. Repeat the wash.
10. After the second wash, remove the supernatant and resuspend the pellet in 20 to 50 µl of Laemmli sample buffer (2% SDS, 10% glycerol, 0.05 M Tris, pH 6.8, containing bromphenol blue as the dye marker)., heat for 5 minutes at 100°C.
11. Microcentrifuge for 5 seconds. Transfer the supernatant to a fresh tube.
12. If a non-reducing gel is to be run, the sample can be loaded directly. If a reducing gel is to be run, add 5% 2-mercaptoethanol, incubate 1 hour 37°C then load sample onto gel.
13. After running the gel, analyze by protein staining, immunoblotting, or autoradiography (if the sample was radiolabeled).

[a] In order to obtain the best results, we recommend trying several amounts of sample to a given amount of beads in order to determine the optimal condition for immunoprecipitation.

Product Profile
Recommended use: 5 µg of Anti-Raf-1 will immuno-precipitate Raf-1 from 50 µl of a cell lysate of Sf9 insect cells expressing human Raf-1 (approximately 10 units of Raf-1).

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

1. Leevers, S.J., Paterson, H.F. and Marshall, C.J., Nature **369**, 411 (1994).
2. Stokoe, D., Macdonald, S.G., Cadwallader, K., Symons, M. and Hancock, J.F., Science **264**, 1463 (1994).
3. Fabian, J.R. Vojtek, A.B., Cooper, J.A. and Morrison, D.K., Proc. Natl. Acad. Sci. USA **91**, 5982 (1994).
4. Brtva, T.R., Drugan, J.K., Ghosh, S., Terrell, R.S. Campbell-Burk, S., Bell, R.M. and Der, C.J. J. Biol. Chem., **270**, 9809 (1995).
5. Dent, P., Jelinek, T., Morrison, D.K., Weber, M.J. and Sturgill, T.W., Science **268**, 1902 (1995).
6. Daum, G., Eisenmann-Tappe, I., Fries, H.-W., Troppmair, J. and Rapp, U.R., Trends Biochem. Sci., **19**, 474 (1994).
7. Sturgill, T.W., Ray, L.B., Erikson, E. and Maller, J.L. Nature **334**, 715 (1988).
8. Waskiewicz, A.J., Flynn, A.F., Proud, C.G. and Cooper, J.A. EMBO J, **16**, 1090 (1997).
9. Wang, X., Flynn, A.F., Waskiewicz, A.J., Webb, B.L.J., Vries, R.G., Baines, I.A., Cooper, J.A., and Proud, C.G. J. Biol. Chem., **273**, 9373 (1998).

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