ProductInformation

ANTI-B-RAF
Developed in Sheep, Affinity Isolated Antibody

Product Number **R7898**

Product Description

Anti-B-Raf is developed in sheep using a synthetic peptide (GGAEPGQALFNGD) that corresponds to the N-terminus of human B-Raf (amino acids 10-22) as immunogen. Affinity isolated antibody is obtained by peptide immunoaffinity chromatography.

Anti-B-Raf specifically reacts with human B-Raf (98 kD) and with several other molecular weight bands (38 kD, 92 kD and 116 kD) but not Raf-A or Raf-1. It is thought that additional bands are either degradation products or modified forms of B-Raf. The antibody cross-reacts with mouse, rat and hamster. The antibody does not interfere with kinase activity. By immunoprecipitation, the antibody immunoprecipitates B-Raf from recombinant human B-Raf expressed in Sf9 insect cells. The enzyme immunocomplex was then used in a coupled phosphorylation assay to activate Mek1 that activated the MAPK-2/Erk2, which phosphorylated the MBP substrate *in vitro*.

Anti-B-Raf may be used for immunoprecipitation of B-Raf.

B-Raf is a proto-oncogene that encodes multiple protein isoforms. At least 10 isoforms of B-Raf have been identified in adult mouse tissues in the ranges of 69-72 kD and 79-99 kD.[1] B-Raf is highly expressed in brain tissues[2] and is activated by Ras[3,4]. The 93-95 kD isoforms show serine/threonine kinase activity that can activate MEK1 in murine NIH 3T3 fibroblasts[5] and rat PC12 cells[6] which then actives Erks. High levels of cAMP have been reported to inhibit both B-Raf[5] and Raf-1.[6,7] 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 been shown that B-Raf can be activated by the small G protein Rap1 both *in vitro*[8] and *in vivo*[9] via a Ras-independent pathway. B-Raf activation initiates an Erk pathway that activates transcription factors and stimulates neuronal differentiation in PC12 cells.
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 B-Raf 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 phosphate buffered saline, pH 7.4, containing 30% glycerol.

Protein concentration is approximately 0.7 mg/ml by Bradford analysis.

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-B-Raf to a reaction tube.
2. Add 100 µl of a 1:1 protein G-agarose slurry that has been washed in PBS.
3. Add 200 µl of PBS.
4. Incubate for 30 min. to 1 hr. at 4°C.
5. Centrifuge at 14,000 rpm for 15 sec.
6. Remove the supernatant and then wash the protein G-agarose pellet twice with the following: Buffer A (50 mM Tris, pH 7.5, 1 mM EDTA, 1 mM EGTA, 0.5 mM Na3VO4, 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 aprotinin and leupeptin).
7. Resuspend the pellet of washed beads in 100 µl of Buffer A.
8. Add sample containing antigen to the beads.**
9. Add 200 µl of ice-cold PBS.
10. Incubate for 2 hours at 4°C.
11. 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.

12. 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.
13. Microcentrifuge for 5 seconds. Transfer the supernatant to a fresh tube.
14. 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.
15. After running the gel, analyze by protein staining, immunoblotting, or autoradiography (if the sample was radiolabeled).

** 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-B-Raf immunoprecipitates B-Raf from 50 µl of a cell lysate of Sf9 insect cells expressing B-Raf.

At 4 ug/ml, the antibody can detect B-RAF by immunoblotting but often two to three other proteins (38, 92 and 116 kDa) are also detected.

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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