Rabbit (polyclonal) Anti-c-Raf Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number:	44-520G
Lot Number:	See product label
Quantity / Volume:	See product label
Form of Antibody:	Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier.
Preservative:	0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)
Purification:	Purified from rabbit serum by epitope-specific affinity chromatography.
Immunogen:	The antiserum was produced against a chemically synthesized peptide derived from the C-terminal region of human c-Raf. The sequence is conserved in mouse and rat.
Target Summary:	The Raf family of serine/threonine-specific kinases is comprised of three members (A-Raf, B-Raf, and c-Raf) that play a critical role in regulating cell growth and differentiation, and couple growth factor receptor stimulation to nuclear transcription factors via the Ras/mitogen-activated protein kinase (MAPK) pathway. c-Raf kinase (also known as Raf-1) is a key 74 kDa signal transducer of multiple extracellular stimuli that is regulated by several pathways, and that once activated, phosphorylates MEK which in turn phosphorylates ERK. Activation of Raf kinase activity is induced by several events including phosphorylation at several sites and binding of GTP-bound Ras and/or 14-3-3 proteins. The c-Raf antibody is useful as a positive control and for measuring total c-Raf protein levels to compare signals obtained with the c-Raf PSSAs.
Reactivity:	Human and Mouse c-Raf. Rat c-Raf (100% homologous) has not been tested, but is expected to react.
Applications:	The antibody has been used in Western blotting. Previous lots have been used in immunocytochemistry.
Suggested Working Dilutions:	For Western blotting applications, we recommend using the antibody at 0.5-2.0 µg/mL. The optimal antibody concentration should be determined empirically for each specific application.
Storage:	Store at –20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at –20°C. For shipment or short-term storage (up to one week), 2-8°C is sufficient.
Expiration Date:	Expires one year from date of receipt when stored as instructed.
Positive Control Used:	HEK293 overexpressing human c-Raf.

This product is for research use only. Not for use in diagnostic procedures.

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PI44520G (Rev 11/08) DCC-08-1089

Related Products:

Antibodies:

c-Raf [pS^{43}], Cat. # 44-501
c-Raf [pS^{259}], Cat. # 44-502
c-Raf [pS^{621}], Cat. # 44-504G
c-Raf [pSpY$^{338/340}$], Cat. # 44-505G
c-Raf [pYpY$^{340/341}$], Cat. # 44-506G
EGFR [pY^{845}], Cat. # 44-784G
Akt/PKB [pT^{308}], Cat. 44-602G
Akt/PKB [pS^{473}], Cat. # 44-621G
MEK1&2 [pS^{222}], Cat. # 44-452
ERK1&2 [pTpY$^{185/187}$], Cat. # 44-680G
JNK1&2 [pTpY$^{183/185}$], Cat. # 44-682G
p38 [pTpY$^{180/182}$], Cat. # 44-684G

ELISAs:

Akt [pS^{473}], Cat. # KHO0111
Akt [pT^{308}], Cat. # KHO0201
MEK1 [pSpS$^{218/222}$], Cat. # KHO0321
ERK1/2 [pTpY$^{185/187}$], Cat. # KHO0091
JNK1/2 [pTpY$^{183/185}$], Cat. # KHO0131
p38 MAPK [pTpY$^{180/182}$], Cat. # KHO0071

References:

Schulze, A., et al. (2004) The transcriptional response to Raf activation is almost completely dependent on Mitogen-activated Protein Kinase Kinase activity and shows a major autocrine component. Mol. Biol. Cell. 15(7):3450-3463.

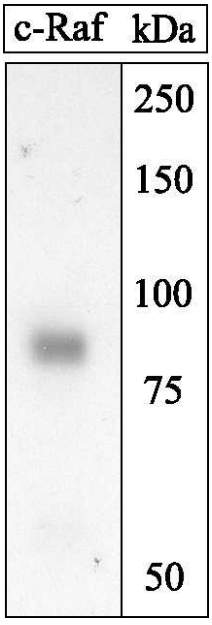
Dhillon, A.S., et al. (2002) Cyclic AMP-dependent protein kinase regulates Raf-1 kinase mainly by phosphorylation of serine 259. Mol. Cell. Biol. 22(10):3237-3246.

Morel, J.C., et al. (2002) Signal transduction pathways involved in rheumatoid arthritis synovial fibroblast interleukin-18-induced vascular cell adhesion molecule-1 expression. J. Biol. Chem. 277(38):34679-34691 (cites the use of cat. # 44-506G, 44-622G and 44-660G).

Yan, F. and D.B. Polk (2001) Kinase suppressor of ras is necessary for tumor necrosis factor alpha activation of extracellular signal-regulated kinase/mitogen-activated protein kinase in intestinal epithelial cells. Cancer Res. 61(3):963-969 (cites the use of cat. # 44-506G).

Gu, M., et al. (2000) Nitric oxide increases p21$^{Waf1/Cip1}$ expression by a cGMP-dependent pathway that includes activation of extracellular signal-regulated kinase and p70^{S6k}. J. Biol. Chem. 275:11389-11396 (cites the use of cat. # 44-504G).

Thorson, J.A., et al. (1998) 14-3-3 Proteins are required for maintenance of Raf-1 phosphorylation and kinase activity. Mol. Cell. Biol. 18:5229-5238 (cites the use of cat. 44-504G).



Western Blot

Extracts of HEK293 cells overexpressing human c-Raf were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF. The membrane was blocked with a 5% BSA-TBST buffer for one hour at room temperature, then incubated with the c-Raf antibody for two hours at room temperature in a 1% BSA-TBST buffer. After washing, the membrane was incubated with goat F(ab')$_2$ anti-rabbit IgG HRP conjugate (Cat. # ALI4404) and signals were detected using the Pierce SuperSignal™ method.

The data show total c-Raf signal in the testing system described above.

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Western Blotting Procedure

1. Lyse approximately 10^7 cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use (Invitrogen catalog number FNN0011). Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifuged at 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of nitrocellulose membrane slightly larger than the gel.
7. Soak the membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) overnight at 4°C or for one hour at room temperature.
12. Incubate the blocked blot with primary antibody at a concentration of 0.5-2.0 µg/mL in Tris buffered saline supplemented with 1% Ig-free BSA and 0.1% Tween 20 overnight at 4°C or for two hours at room temperature.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat $F(ab')_2$ anti-rabbit IgG alkaline phosphatase conjugate (catalog number ALI4405) or goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (catalog number ALI4404) in conjunction with your chemiluminescence reagents and instrumentation.

Cell Lysis Buffer Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM $Na_4P_2O_7$
2 mM Na_3VO_4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO) or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma catalog number P2714 may be used)

Transfer Buffer Formulation:
2.4 gm Tris base
14.2 gm glycine
200 mL methanol
Q.S. to 1 liter, then add
1 mL 10% SDS.
Cool to 4°C prior to use.

Tris Buffered Saline Formulation:
20 mM Tris-HCl, pH 7.4
0.9% NaCl

Blocking Buffer Formulation:
100 mL Tris buffered saline
5 gm Ig-free BSA
0.1 mL Tween 20

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