Background: A-Raf, B-Raf and c-Raf (Raf-1) are the main effectors recruited by GTP-bound Ras to activate the MEK-MAP kinase pathway (1). Activation of c-Raf is the best understood and involves phosphorylation at multiple activating sites including Ser338, Tyr341, Ser494, Ser497 and Ser499 (2). p21-activated protein kinase (PAK) has been shown to phosphorylate c-Raf at Ser338 and the Srf family phospholipases Tyr341 to induce c-Raf activity (3,4). Ser338 of c-Raf corresponds to similar sites in A-Raf (Ser299) and B-Raf (Ser445), although this site is constitutively phosphorylated in B-Raf (5). Inhibitory 14-3-3 binding sites on c-Raf (Ser259 and Ser621) can be phosphorylated by Akt and AMPK, respectively (6,7). While A-Raf, B-Raf and c-Raf are similar in sequence and function, differential regulation has been observed (8). Of particular interest, B-Raf contains three consensus Akt phosphorylation sites (Ser364, Ser428 and Thr439) and lacks a site equivalent to Tyr341 of c-Raf (8,9). The B-Raf mutation V600E results in elevated kinase activity and is commonly found in malignant melanoma (10). Six residues of c-Raf (Ser29, Ser43, Ser289, Ser296, Ser301 and Ser642) become hyperphosphorylated in a manner consistent with c-Raf inactivation. The hyperphosphorylation of these six sites is dependent on downstream MEK signaling and renders c-Raf unresponsive to subsequent activation events (11).

Specificity/Sensitivity: c-Raf Antibody detects endogenous levels of total c-Raf protein.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to a region surrounding Pro302 of human c-Raf. Antibodies are purified by protein A and peptide affinity chromatography.

Applications | Species Cross-Reactivity* | Molecular Wt. | Source
--- | --- | --- | ---
W Endogenous | H, M, R, Mk | 65 to 75 kDa | Rabbit**

Recommended Antibody Dilutions:
Western Blotting: 1:1000

For application specific protocols please see the web page for this product at www.cellsignal.com. Please visit www.cellsignal.com for a complete listing of recommended companion products.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at −20°C. Do not aliquot the antibody.

Species cross-reactivity is determined by western blot.
Anti-rabbit secondary antibodies must be used to detect this antibody.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide

Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mm—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine

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