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## **A-Raf Antibody**



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Applications: Reactivity: Sensitivity: MW (kDa): Source: **UniProt ID:** Entrez-Gene Id: WB, IP  $\mathsf{H}\,\mathsf{M}\,\mathsf{R}$ Endogenous 68 Rabbit #P10398 369 **Product Usage** Application Dilution Information Western Blotting 1:1000 Immunoprecipitation 1:50 Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μg/ml BSA and 50% glycerol. Store at -**Storage** 20°C. Do not aliquot the antibody. Specificity / Sensitivity A-Raf Antibody detects endogenous levels of total A-Raf. This antibody does not cross-react with c-Raf or Source / Purification Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues close to the linker domain of human A-Raf. Antibodies are purified by protein A and peptide affinity chromatography.

**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, Thr491, Ser494, Ser497, and Ser499 (2). p21-activated kinase (PAK) has been shown to phosphorylate c-Raf at Ser338, and the Src family phosphorylates 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). Research studies have shown that 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).

## **Background References**

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- 6. Zimmermann, S. and Moelling, K. (1999) Science 286, 1741-4.
- 7. Sprenkle, A.B. et al. (1997) FEBS Lett 403, 254-8.
- 8. Marais, R. et al. (1997) J Biol Chem 272, 4378-83.
- 9. Guan, K.L. et al. (2000) J Biol Chem 275, 27354-9.
- Davies, H. et al. (2002) Nature 417, 949-54.
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**Species Reactivity** 

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

**Western Blot Buffer** 

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

Applications Key

WB: Western Blotting IP: Immunoprecipitation

**Cross-Reactivity Key** 

A-Raf Antibody (#4432) Datasheet Without Images Cell Signaling Technology

H: human M: mouse R: rat Hm: hamster Mk: monkey Vir: virus Mi: mink C: chicken Dm: D. melanogaster X: Xenopus Z: zebrafish B: bovine Dg: dog Pg: pig Sc: S. cerevisiae Ce: C. elegans Hr: horse GP: Guinea Pig Rab: rabbit All: all species expected

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