

Phospho-A-Raf (Ser299) Antibody

✓ 100 µl
(10 western blots)

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

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Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Endogenous	H, M, R	68 kDa	Rabbit**

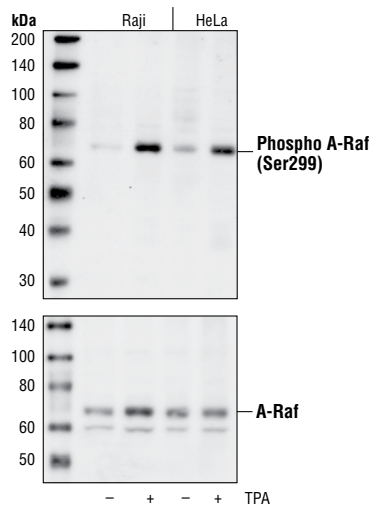
Background: A-Raf, B-Raf and c-Raf (Raf-1) are the main effectors recruited by GTP-bound Ras to activate the MEK-MAP kinase pathway (1). Activation of c-Raf is the best understood and involves phosphorylation at multiple activating sites including Ser338, Tyr341, Thr491, Ser494, Ser497 and Ser499 (2). p21-activated protein 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). 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: Phospho-A-Raf (Ser299) Antibody detects endogenous levels of A-Raf only when phosphorylated at Ser299. The antibody does not cross-react with B-Raf or Raf-1 under physiological conditions.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Ser299 of human A-Raf. Antibodies are purified by protein A and peptide affinity chromatography.

Background References:

- (1) Avruch, J. et al. (1994) *Trends Biochem. Sci.* 19, 279–283.
- (2) Chong, H. et al. (2001) *EMBO J.* 20, 3716–3727.
- (3) King, A.J. et al. (1998) *Nature* 396, 180–183.
- (4) Fabian, J.R. et al. (1993) *Mol. Cell Biol.* 13, 7170–7179.
- (5) Mason, C.S. et al. (1999) *EMBO J.* 18, 2137–2148.
- (6) Zimmermann, S. and Moelling, K. (1999) *Science* 286, 1741–1744.
- (7) Sprenkle, A.B. et al. (1997) *FEBS Lett.* 403, 254–258.
- (8) Marais, R. et al. (1997) *J. Biol. Chem.* 272, 4378–4383.
- (9) Guan, K.L. et al. (2000) *J. Biol. Chem.* 275, 27354–27359.
- (10) Davies, H. et al. (2002) *Nature* 417, 949–954.
- (11) Dougherty, M.K. et al. (2005) *Mol. Cell* 17, 215–224.



Western blot analysis of extracts from Raji and HeLa cells, untreated or TPA-treated (30 minutes) using Phospho-A-Raf (Ser299) Antibody (upper) or A-Raf Antibody, #4432 (lower).

Entrez-Gene ID #369
Swiss-Prot Acc. #P10398

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.

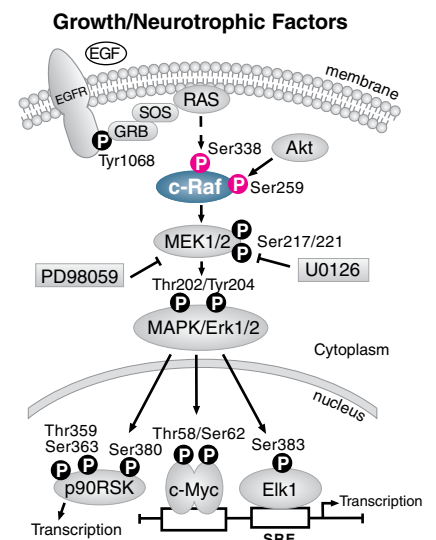
**Anti-rabbit secondary antibodies must be used to detect this antibody.

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.



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 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 All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.