

#9421 Store at -20°C

# Phospho-c-Raf (Ser259) Antibody

100 µl  
 (10 Western mini-blot)

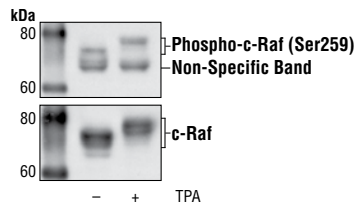
**Orders** ■ 877-616-CELL (2355)  
 orders@cellsignal.com  
**Support** ■ 877-678-TECH (8324)  
 info@cellsignal.com  
**Web** ■ www.cellsignal.com

rev 01/14/09

This product is for *in vitro* research use only and is not intended for use in humans or animals.  
 This product is not intended for use as a therapeutic or in diagnostic procedures.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP Endogenous	H, M, R, C, X	74 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).



Western blot analysis of extracts from HeLa cells, untreated or TPA-treated, using Phospho-c-Raf (Ser259) Antibody (upper), or a total c-Raf antibody (lower).

**Specificity/Sensitivity:** Phospho-c-Raf (Ser259) Antibody detects endogenous levels of c-Raf only when phosphorylated at Ser259.

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Ser259 of human c-Raf. Antibodies are purified by protein A and peptide affinity chromatography.

Entrez-Gene ID # 5894  
 Swiss-Prot Acc. # P04049

**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.

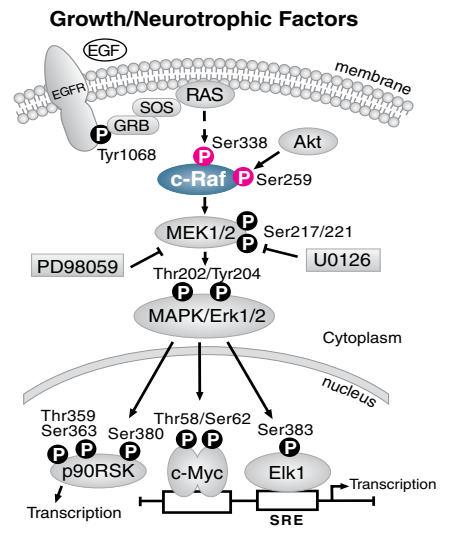
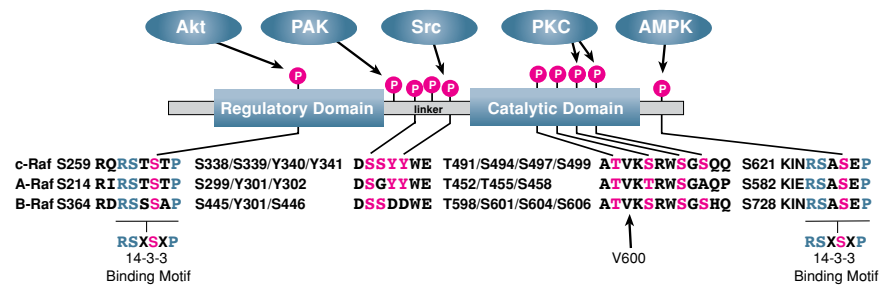
\*Species cross-reactivity is determined by Western blot.  
\*\*Anti-rabbit secondary antibodies must be used to detect this antibody.

**Recommended Antibody Dilutions:**  
 Western blotting 1:1000  
 Immunoprecipitation 1:50

For application specific protocols please see the web page for this product at [www.cellsignal.com](http://www.cellsignal.com).

**Companion Products:**  
 Phospho-MEK1/2 (Ser217/221) Antibody #9121  
 MEK1/2 Antibody #9122  
 Phospho-(Ser) 14-3-3 Binding Motif Antibody #9601  
 PD98059 (MEK1 Inhibitor) #9900  
 LY294002 (PI3 Kinase Inhibitor) #9901

Please visit [www.cellsignal.com](http://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—zebra fish B—bovine  
 Dg—dog Pg—pig Sc—S. cerevisiae All—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

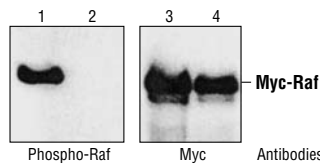
### Selected Application References:

Cieslik, K. et al. (2001) Upregulation of endothelial nitric oxide synthase promoter by PI3K( $\gamma$ )/Jak2/MEK-1-dependent pathway. *J. Biol. Chem.* 276, 1211–1219. Application: W.

Weber, S.M. et al. (2002) Inhibition of mitogen-activated protein kinase signaling by chloroquine. *J. Immunol.* 168, 5303–5309. Application: W.

### 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) Sprengle, 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.



*Site specificity of Phospho-c-Raf (Ser259) Antibody: Western blot analysis of recombinant Myc-tagged c-Raf protein, wild-type (lanes 1 and 3) and S259A mutant (lanes 2 and 4), using Phospho-Raf (Ser259) Antibody or a Myc antibody. (Provided by Dr. Guri Tzivion, Massachusetts General Hospital.)*