Store at -20°C

#9427

Phospho-c-Raf (Ser338) (56A6) Rabbit mAb

✓ 100 µl
(10 Western mini-blots)

Cell Signaling TECHNOLOGY®

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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.

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

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W Endogenous	H, M, R, Mk	74 kDa	Rabbit IgG**

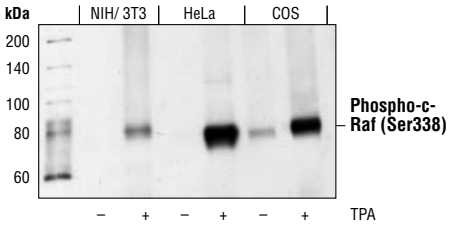
Background: A-Raf, B-Raf and c-Raf (Raf-1) are the main effectors recruited by GTP-bound Ras to activate the MEK-MAP kinase pathway (1). Activation of c-Raf is the best understood and involves phosphorylation at multiple activating sites including Ser338, Tyr341, Thr491, Ser494, Ser497 and Ser499 (2). 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-c-Raf (Ser338) (56A6) Rabbit mAb detects endogenous levels of c-Raf only when phosphorylated at Ser338.

Source/Purification: Monoclonal antibody is produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding serine 338 of human Raf.

Selected Application References:

Kajiya, M. et al. (2008) Brain-derived neurotrophic factor stimulates bone/cementum-related protein gene expression in cementoblasts. *J Biol Chem* 283, 16259–67. Application: W.



Western blot analysis of extracts from NIH3T3, HeLa and COS cells, untreated or treated with TPA, using Phospho-c-Raf (Ser338) (56A6) Rabbit mAb.

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.

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.

Companion Products:

Phospho-c-Raf (Ser259) Antibody #9421
c-Raf Antibody #9422
Phospho-A-Raf (Ser299) Antibody #4431
A-Raf Antibody #4432
Phospho-B-Raf (Ser445) Antibody #2696
Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
Anti-rabbit IgG, HRP-linked Antibody #7074
estained Protein Marker, Broad Range (Premixed Format) #7720
Biotinylated Protein Ladder Detection Pack #7727
20X LumiGLO® Reagent and 20X Peroxide #7003

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

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