

# p-Raf-1 (Ser 301): sc-135707

## BACKGROUND

Raf-1 is a ubiquitously expressed cytoplasmic protein with intrinsic serine/threonine kinase activity. Raf-1, or c-Raf, is the cellular homolog of v-Raf, the product of the transforming gene of the 3611 strain of murine sarcoma virus. The unregulated kinase activity of the v-Raf protein is associated with cellular transformation and mitogenesis. Raf-1 is normally suppressed by its regulatory N-terminal domain. Raf-1 is activated in response to a variety of tyrosine kinase receptors as well as in response to pp60v-Src expression. Specifically, Raf-1 is phosphorylated in the catalytic domain at Ser 338 and, to a lesser extent, Ser 339. This phosphorylation requires the co-activation of PI 3-kinase and the Ras signaling pathway. Raf-1 is also phosphorylated on Tyr 340 and 341, which induces the phosphorylation of MEK. Phosphorylation of Ser 621 is essential for the catalytic activity of Raf-1 and downregulation by c-AMP-dependent protein kinase A (PKA). PKA also phosphorylates Raf-1 on Ser 43 and Ser 259. PKA phosphorylation of Ser 259 inhibits Raf-1 and decreases the phosphorylation necessary for Raf-1 activation at Ser 338.

## REFERENCES

1. Rapp, U.R., et al. 1983. Structure and biological activation of v-Raf, a unique oncogene transduced by a retrovirus. *Proc. Natl. Acad. Sci. USA* 80: 4218-4222.
2. Huleihel, M., et al. 1986. Characterization of murine A-Raf, a new oncogene related to the v-Raf oncogene. *Mol. Cell. Biol.* 6: 2655-2662.
3. Heidecker, G., et al. 1990. Mutational activation of c-Raf-1 and definition of the minimal transforming sequence. *Mol. Cell. Biol.* 10: 2503-2512.
4. Mischak, H., et al. 1996. Negative regulation of Raf-1 by phosphorylation of Serine 621. *Mol. Cell. Biol.* 16: 5409-5418.
5. Diaz, B., et al. 1997. Phosphorylation of Raf-1 Serine 338-Serine 339 is an essential regulatory event for Ras-dependent activation and biological signaling. *Mol. Cell. Biol.* 17: 4509-4516.
6. King, A.J., et al. 1998. The protein kinase PAK3 positively regulates Raf-1 activity through phosphorylation of Serine 338. *Nature* 396: 180-183.
7. Barnard, D., et al. 1998. Oncogenes, growth factors and phorbol esters regulate Raf-1 through common mechanisms. *Oncogene* 17: 1539-1547.

## CHROMOSOMAL LOCATION

Genetic locus: RAF1 (human) mapping to 3p25.2; Raf1 (mouse) mapping to 6 E3.

## SOURCE

p-Raf-1 (Ser 301) is a rabbit polyclonal antibody raised against a short amino acid sequence containing Ser 301 phosphorylated Raf-1 of rat origin.

## PRODUCT

Each vial contains IgG in 100  $\mu$ l of 10 mM HEPES with 150 mM NaCl, 50% glycerol and < 0.1% BSA.

## APPLICATIONS

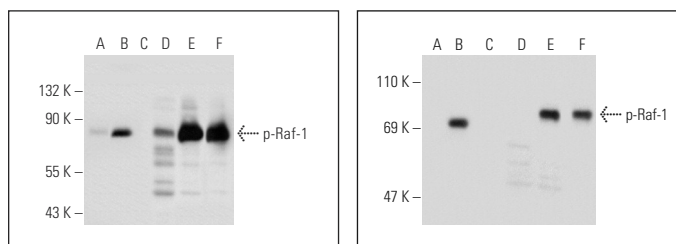
p-Raf-1 (Ser 301) is recommended for detection of Ser 301 phosphorylated Raf-1 of mouse, rat, human, *Xenopus*, bovine and canine origin by Western Blotting (starting dilution to be determined by researcher, dilution range 1:100-1:5000) and immunoprecipitation [1-2  $\mu$ l per 100-500  $\mu$ g of total protein (1 ml of cell lysate)].

Suitable for use as control antibody for Raf-1 siRNA (h): sc-29462, Raf-1 siRNA (m): sc-29463, Raf-1 shRNA Plasmid (h): sc-29462-SH, Raf-1 shRNA Plasmid (m): sc-29463-SH, Raf-1 shRNA (h) Lentiviral Particles: sc-29462-V and Raf-1 shRNA (m) Lentiviral Particles: sc-29463-V.

Molecular Weight of p-Raf-1: 74 kDa.

Positive Controls: Raf-1 (m): 293T Lysate: sc-122942, Raf-1 (h): 293 Lysate: sc-158911 or CCD-1064Sk + PDGF cell lysate: sc-2264.

## DATA



Western blot analysis of Raf-1 phosphorylation in non-transfected: sc-117752 (A,D), untreated mouse Raf-1 transfected: sc-122942 (B,E) and lambda protein phosphatase treated mouse Raf-1 transfected: sc-122942 (C,F) 293T whole cell lysates. Antibodies tested include p-Raf-1 (Ser 301): sc-135707 (A,B,C) and Raf-1 (540): sc-52827 (D,E,F).

Western blot analysis of Raf-1 phosphorylation in non-transfected: sc-110760 (A,D), untreated human Raf-1 transfected: sc-158911 (B,E) and lambda protein phosphatase (sc-200312A) treated human Raf-1 transfected: sc-158911 (C,F) 293 whole cell lysates. Antibodies tested include p-Raf-1 (Ser 301): sc-135707 (A,B,C) and Raf-1 (540): sc-52827 (D,E,F).

## STORAGE

For immediate and continuous use, store at 4° C for up to one month. For sporadic use, freeze in working aliquots in order to avoid repeated freeze/thaw cycles. If turbidity is evident upon prolonged storage, clarify solution by centrifugation.

## RESEARCH USE

For research use only, not for use in diagnostic procedures.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog for detailed protocols and support products.