

# p-Raf-1 (Ser 621)-R: sc-16807-R

## BACKGROUND

Raf-1 is a 72 to 76 kDa 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 down-regulation 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.

## CHROMOSOMAL LOCATION

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

## SOURCE

p-Raf-1 (Ser 621)-R is a rabbit polyclonal antibody raised against a short amino acid sequence containing phosphorylated Ser 621 of Raf-1 of human origin.

## PRODUCT

Each vial contains 200 µg IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-16807 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% BSA).

## STORAGE

Store at 4° C, **\*\*DO NOT FREEZE\*\***. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

## 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.

## APPLICATIONS

p-Raf-1 (Ser 621)-R is recommended for detection of Ser 621 phosphorylated Raf-1 of mouse, rat, human and zebrafish origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

p-Raf-1 (Ser 621)-R is also recommended for detection of correspondingly phosphorylated Ser on Raf-1 in additional species, including equine, canine, bovine, porcine and avian.

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.

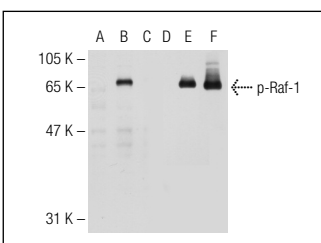
Molecular Weight of p-Raf-1: 74 kDa.

Positive Controls: Raf-1 (m): 293T Lysate: sc-122942, HeLa + UV irradiated cell lysate: sc-2221 or HeLa + PMA/PE whole cell lysate: sc-24808.

## RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent), Western Blotting Luminol Reagent: sc-2048 and Lambda Phosphatase: sc-200312A. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## 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 (sc-200312A) treated mouse Raf-1 transfected: sc-122942 (C,F) 293T whole cell lysates. Antibodies tested include p-Raf-1 (Ser 621)-R: sc-16807-R (A,B,C) and Raf-1 (540): sc-52827 (D,E,F).

## SELECT PRODUCT CITATIONS

1. Noble, C., et al. 2008. CRAF autophosphorylation of Serine 621 is required to prevent its proteasome-mediated degradation. Mol. Cell 31: 862-872.