SANTA CRUZ BIOTECHNOLOGY, INC.

p-Raf-1 (Ser 259)-R: sc-21833-R

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

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.

CHROMOSOMAL LOCATION

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

SOURCE

p-Raf-1 (Ser 259)-R is a rabbit polyclonal antibody raised against a short amino acid sequence containing phosphorylated Ser 259 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-21833 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.

APPLICATIONS

p-Raf-1 (Ser 259)-R is recommended for detection of Ser 259 phosphorylated Raf-1 of mouse, rat and human 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 259)-R is also recommended for detection of correspondingly phosphorylated Ser on Raf-1 in additional species, including equine, canine, bovine and porcine.

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.

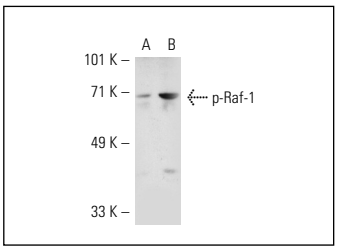
Molecular Weight of p-Raf-1: 74 kDa.

Positive Controls: HeLa whole cell lysate: sc-2200, HeLa + UV irradiated cell lysate: sc-2221 or Raf-1 (h): 293 Lysate: sc-113164.

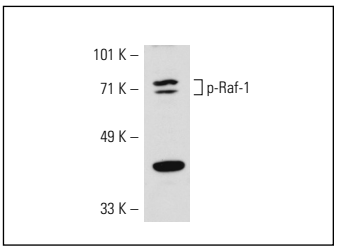
RECOMMENDED SECONDARY REAGENTS

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) and Western Blotting Luminol Reagent: sc-2048. 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



p-Raf-1 (Ser 259)-R: sc-21833-R. Western blot analysis of phosphorylated Raf-1 expression in non-transfected: sc-117752 (**A**) and human Raf-1 transfected: sc-113164 (**B**) 293T whole cell lysates.



p-Raf-1 (Ser 259)-R: sc-21833-R. Western blot analysis of phosphorylated Raf-1 expression in KNRK whole cell lysate.

RESEARCH USE

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

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.