SANTA CRUZ BIOTECHNOLOGY, INC.

p-Raf-1 (Ser 338/Tyr 341)-R: sc-28005-R

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

Raf-1 is an 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 acti-vation 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 mech-anisms. Oncogene 17: 1539-1547.
8. Chaudhary, A., et al. 2000. Phosphatidylinositol 3-kinase regulates Raf-1 through PAK phosphorylation of Serine 338. Curr. Biol. 10: 551-554.

CHROMOSOMAL LOCATION

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

SOURCE

p-Raf-1 (Ser 338/Tyr 341)-R is a rabbit polyclonal antibody raised against a short amino acid sequence containing phosphorylated Ser 338 and Tyr 341 of Raf-1 of human origin.

STORAGE

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

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-28005 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% BSA).

APPLICATIONS

p-Raf-1 (Ser 338/Tyr 341)-R is recommended for detection of Ser 338 and Tyr 341 dually 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).

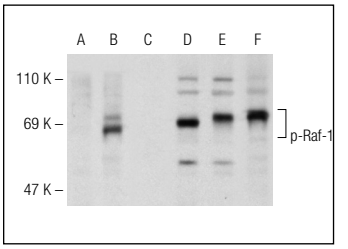
p-Raf-1 (Ser 338/Tyr 341)-R is also recommended for detection of correspondingly phosphorylated Ser and Tyr on Raf-1 in additional species, including equine, canine, bovine and avian.

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: 80 kDa.

Positive Controls: HeLa whole cell lysate: sc-2200, HeLa + PMA/PE whole cell lysate: sc-24808 or CCD-1064Sk cell lysate: sc-2263.

DATA



Western blot analysis of Raf-1 phosphorylation in untreated (**A**,**D**), serum starved and PMA treated (**B**,**E**) and serum starved, PMA treated and lambda protein phosphatase (sc-200312A) treated (**C**,**F**) HeLa whole cell lysates. Antibodies tested include p-Raf-1 (Ser 338/Tyr 341)-R: sc-28005-R (**A**,**B**,**C**) and Raf-1 (C-20): sc-227 (**D**,**E**,**F**).

SELECT PRODUCT CITATIONS

1. Sánchez, Y., et al. 2009. Regulation of genistein-induced differentiation in human acute myeloid leukaemia cells (HL60, NB4) protein kinase modulation and reactive oxygen species generation. Biochem. Pharmacol. 77: 384-396.

RESEARCH USE

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