SANTA CRUZ BIOTECHNOLOGY, INC.

p-Raf-1 (Ser 259): sc-101791

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.

CHROMOSOMAL LOCATION

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

SOURCE

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

PRODUCT

Each vial contains 100 µg IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

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) 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 immunohistochemistry (including paraffin-embedded sections) (starting dilution 1:50, dilution range 1:50-1:500).

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.

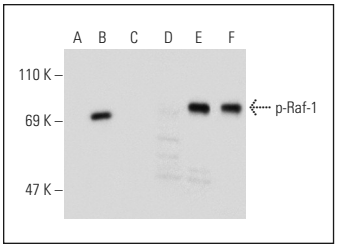
Molecular Weight of p-Raf-1: 74 kDa.

Positive Controls: NIH/3T3 whole cell lysate: sc-2210, KNRK whole cell lysate: sc-2214 or Jurkat whole cell lysate: sc-2204.

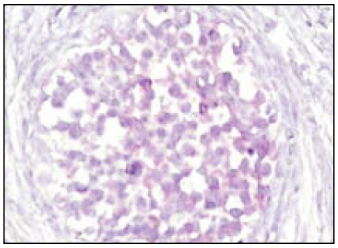
RECOMMENDED SECONDARY REAGENTS

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. 4) Immunohistochemistry: use ImmunoCruz™: sc-2051 or ABC: sc-2018 rabbit IgG Staining Systems.

DATA



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 259): sc-101791 (**A**,**B**,**C**) and Raf-1 (540): sc-52827 (**D**,**E**,**F**).



p-Raf-1 (Ser 259): sc-101791. Immunoperoxidase staining of formalin-fixed, paraffin-embedded human breast carcinoma tissue showing nuclear staining.

SELECT PRODUCT CITATIONS

1. El-Naggar, S., et al. 2009. Mutation of the Rb1 pathway leads to overexpression of mTor, constitutive phosphorylation of Akt on serine 473, resistance to anoikis, and a block in c-Raf activation. Mol. Cell. Biol. 29: 5710-5717.
2. Gu, H., et al. 2015. Mitochondrial E3 ligase March5 maintains stemness of mouse ES cells via suppression of ERK signalling. Nat. Commun. 6: 7112.

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.