

p-Raf-B (Thr 598/Ser 601): sc-28006

BACKGROUND

Several Serine/Threonine protein kinases have been implicated as intermediates in signal transduction pathways. These include ERK/MAP kinases, ribosomal S6 kinase (Rsk) and Raf-1. Raf-1 is a 72 to 76 kDa cytoplasmic protein with intrinsic Serine/Threonine activity. It is broadly expressed in nearly all cell lines tested to date and 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 has been associated with transformation and mitogenesis while the activity of Raf-1 is normally suppressed by a regulatory N-terminal domain. Raf-A, a second member of the Raf gene family of Serine/Threonine protein kinases, exhibits substantial homology to Raf-1 within the kinase domain of the two molecules, but less homology elsewhere. Expression of Raf-B is highly restricted with highest levels in the cerebrum and testes.

REFERENCES

- 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.
- 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.
- Sariban, E., et al. 1987. Expression of the c-Raf protooncogene in human hematopoietic cells and cell lines. *Blood* 69: 1437-1440.
- Ray, L.B., et al. 1988. Insulin-stimulated microtubule-associated protein kinase is phosphorylated on tyrosine and Threonine *in vivo*. *Proc. Natl. Acad. Sci. USA* 85: 3753-3757.
- Morrison, D.K., et al. 1988. Signal transduction from membrane to cytoplasm: growth factors and membrane-bound oncogene products increase Raf-1 phosphorylation and associated protein kinase activity. *Proc. Natl. Acad. Sci. USA* 85: 8855-8859.
- Pelech, S.L., et al. 1990. Protein kinase cascades in meiotic and mitotic cell cycle control. *Biochem. Cell Biol.* 68: 1297-1330.
- Heidecker, G., et al. 1990. Mutational activation of c-Raf-1 and definition of the minimal transforming sequence. *Mol. Cell. Biol.* 10: 2503-2512.
- Storm, S.M., et al. 1990. Expression of raf family proto-oncogenes in normal mouse tissues. *Oncogene* 5: 345-351.
- Turner, B., et al. 1991. Interleukin 2 induces tyrosine phosphorylation and activation of p72-74 Raf-1 kinase in a T cell line. *Proc. Natl. Acad. Sci. USA* 88: 1227-1231.

CHROMOSOMAL LOCATION

Genetic locus: BRAF (human) mapping to 7q34; Braf (mouse) mapping to 6 B1.

SOURCE

p-Raf-B (Thr 598/Ser 601) is available as either a goat (sc-28006) or rabbit (sc-28006-R) polyclonal affinity purified antibody raised against a short amino acid sequence containing phosphorylated Thr 598 and Ser 601 of Raf-B 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-28006 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% BSA).

APPLICATIONS

p-Raf-B (Thr 598/Ser 601) is recommended for detection of Thr 598 and Ser 601 dually phosphorylated Raf-B of mouse, rat and human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), 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).

Suitable for use as control antibody for Raf-B siRNA (h): sc-36368.

Molecular Weight of p-Raf-B: 95/62 kDa.

RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: for goat primary antibody (sc-28006): use donkey anti-goat IgG-HRP: sc-2020 (range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (range: 1:2000-1:5000), for rabbit primary antibody (sc-28006-R): use goat anti-rabbit IgG-HRP: sc-2004 (range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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) Immunofluorescence: for goat primary antibody (sc-28006): use donkey anti-goat IgG-FITC: sc-2024 (range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (range: 1:100-1:400), for rabbit primary antibody (sc-28006-R): use goat anti-rabbit IgG-FITC: sc-2012 (range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

SELECT PRODUCT CITATIONS

- Lissitzky, J.C., et al. 2009. Cyclic AMP signaling as a mediator of vasculogenic mimicry in aggressive human melanoma cells *in vitro*. *Cancer Res.* 69: 802-809.
- Mikula, M., et al. 2011. Direct recruitment of ERK cascade components to inducible genes is regulated by heterogeneous nuclear ribonucleoprotein (hnRNP) K. *J. Biol. Chem.* 286: 9763-9775.

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 or our catalog for detailed protocols and support products.