

Raf-1 (h): 293T Lysate: sc-158911

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 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 3,611 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-1 is activated in response to activation of a variety of tyrosine kinase receptors as well as in response to pp60v-Src expression. There is accumulating evidence that Ras p21 may play a role in activation of Raf-1 and may play the role of the messenger from membrane tyrosine kinases to Raf-1.

REFERENCES

1. Rapp, U.R. Goldsborough, M.D., Mark, G.E., Bonner, T.I., Groffen, J., Reynolds, F.H. and Stephenson, J.R. 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., Goldsborough, M., Cleveland, J.L., Gunnell, M., Bonner, T. and Rapp, U.R. 1986. Characterization of murine A-Raf, a new oncogene related to the v-Raf oncogene. *Mol. Cell. Biol.* 6: 2655-2662.
3. Ray, L.B. and Sturgill, T.W. 1988. Insulin-stimulated microtubule-associated protein kinase is phosphorylated on tyrosine and threonine *in vivo*. *Proc. Natl. Acad. Sci. USA* 85: 3753-3757.
4. Morrison, D.K., Kaplan, D.R., Rapp, U. and Roberts, T.M. 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.
5. Pelech, S.L., Sanghera, J.S. and Daya-Makin, M. 1990. Protein kinase cascades in meiotic and mitotic cell cycle control. *Biochem. Cell Biol.* 68: 1297-1330.
6. Heidecker, G., Huleihel, M., Cleveland, J.L., Kolch, W., Beck, P., Lloyd, P., Pawson, T. and Rapp, U.R. 1990. Mutational activation of c-Raf-1 and definition of the minimal transforming sequence. *Mol. Cell. Biol.* 10: 2503-2512.
7. Turner, B., Rapp, U., App, H., Greene, M., Dobashi, K. and Reed, J. 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.
8. Williams, N.G., Roberts, T.M. and Li, P. 1992. Both p21Ras and pp60v-Src are required, but neither alone is sufficient, to activate the Raf-1 kinase. *Proc. Natl. Acad. Sci. USA* 89: 2922-2926.

CHROMOSOMAL LOCATION

Genetic locus: RAF1 (human) mapping to 3p25.2.

STORAGE

Store at -20° C. Repeated freezing and thawing should be minimized. Sample vial should be boiled once prior to use. Non-hazardous. No MSDS required.

PRODUCT

Raf-1 (h): 293T Lysate represents a lysate of human Raf-1 transfected 293T cells and is provided as 100 µg protein in 200 µl SDS-PAGE buffer.

APPLICATIONS

Raf-1 (h): 293T Lysate is suitable as a Western Blotting positive control for human reactive Raf-1 antibodies. Recommended use: 10-20 µl per lane.

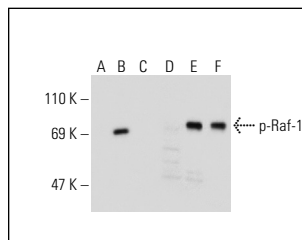
Control 293T Lysate: sc-117752 is available as a Western Blotting negative control lysate derived from non-transfected 293 cells.

Raf-1 (540): sc-52827. is recommended as a positive control antibody for Western Blot analysis of enhanced human Raf-1 expression in Raf-1 transfected 293 cells (starting dilution 1:100, dilution range 1:100-1:1,000).

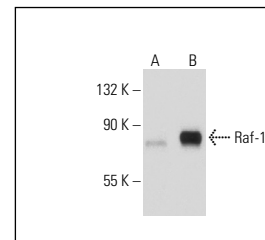
RECOMMENDED SUPPORT REAGENTS

To ensure optimal results, the following support reagents are recommended:
1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048.

DATA



Western blot analysis of Raf-1 phosphorylation in non-transfected: sc-117752 (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) 293T 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).



Raf-1 (540): sc-52827. Western blot analysis of Raf-1 expression in non-transfected: sc-117752 (A) and human Raf-1 transfected: sc-158911 (B) 293T whole cell lysates.

RESEARCH USE

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

PROTOCOLS

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