SANTA CRUZ BIOTECHNOLOGY, INC.

A-Raf siRNA (m): sc-29616

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 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. A-Raf, 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 A-Raf is found at highest levels in urogenital tissues and kidney and at lowest level in brain tissue.

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. Sariban, E., et al. 1987. Expression of the c-raf protooncogene in human hematopoietic cells and cell lines. Blood 69: 1437-1440.
4. 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.
5. 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.
6. Pelech, S.L., et al. 1990. Protein kinase cascades in meiotic and mitotic cell cycle control. Biochem. Cell Biol. 68: 1297-1330.
7. Heidecker, G., et al. 1990. Mutational activiation of c-Raf-1 and definition of the minimal transforming sequence. Mol. Cell. Biol. 10: 2503-2512.

CHROMOSOMAL LOCATION

Genetic locus: Araf (mouse) mapping to X A1.3.

PRODUCT

A-Raf siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see A-Raf shRNA Plasmid (m): sc-29616-SH and A-Raf shRNA (m) Lentiviral Particles: sc-29616-V as alternate gene silencing products.

For independent verification of A-Raf (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-29616A, sc-29616B and sc-29616C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

A-Raf siRNA (m) is recommended for the inhibition of A-Raf expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

A-Raf (A-5): sc-166771 is recommended as a control antibody for monitoring of A-Raf gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

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. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor A-Raf gene expression knockdown using RT-PCR Primer: A-Raf (m)-PR: sc-29616-PR (20 µl, 521 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.