

A-Raf siRNA (h): sc-29615

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. 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.

CHROMOSOMAL LOCATION

Genetic locus: ARAF (human) mapping to Xp11.23.

PRODUCT

A-Raf siRNA (h) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see A-Raf shRNA Plasmid (h): sc-29615-SH and A-Raf shRNA (h) Lentiviral Particles: sc-29615-V as alternate gene silencing products.

For independent verification of A-Raf (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-29615A, sc-29615B and sc-29615C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

A-Raf siRNA (h) is recommended for the inhibition of A-Raf expression in human 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 μ M in 66 μ l. 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.

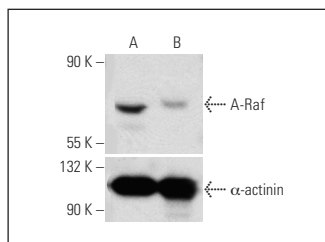
GENE EXPRESSION MONITORING

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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor A-Raf gene expression knockdown using RT-PCR Primer: A-Raf (h)-PR: sc-29615-PR (20 μ l, 470 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

DATA



A-Raf siRNA (h): sc-29615. Western blot analysis of A-Raf expression in non-transfected control (A) and A-Raf transfected (B) HeLa cells. Blot probed with A-Raf (C-20): sc-408. α -actinin (H-2): sc-17829 used as specificity and loading control.

SELECT PRODUCT CITATIONS

1. Gunawardhana, N., et al. 2017. *Helicobacter pylori*-induced HB-EGF upregulates gastrin expression via the EGF receptor, C-Raf, Mek1, and Erk2 in the MAPK pathway. *Front. Cell. Infect. Microbiol.* 7: 541.

RESEARCH USE

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