SANTA CRUZ BIOTECHNOLOGY, INC.

Raf-B siRNA (h): sc-36368

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The Power to Question

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-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 testis.

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: BRAF (human) mapping to 7q34.

PRODUCT

Raf-B siRNA (h) is a target-specific 19-25 nt siRNA 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 Raf-B shRNA Plasmid (h): sc-36368-SH and Raf-B shRNA (h) Lentiviral Particles: sc-36368-V as alternate gene silencing products.

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

Raf-B siRNA (h) is recommended for the inhibition of Raf-B 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 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

Raf-B (F-7): sc-5284 is recommended as a control antibody for monitoring of Raf-B 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 Raf-B gene expression knockdown using RT-PCR Primer: Raf-B (h)-PR: sc-36368-PR (20 µl, 434 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. McCarty, S.K., et al. 2014. BRAF activates and physically interacts with PAK to regulate cell motility. Endocr. Relat. Cancer 21: 865-877.
2. Lankenau, M.A., et al. 2015. MicroRNA-3151 inactivates TP53 in BRAF-mutated human malignancies. Proc. Natl. Acad. Sci. USA 112: E6744-E6751.
3. Wang, X., et al. 2017. BRAF signals to pro-apoptotic BIM to enhance AraC cytotoxicity induced in AML cells by vitamin D-based differentiation agents. J. Steroid Biochem. Mol. Biol. 173: 139-147.
4. Schmidlin, C.J., et al. 2021. FAM129B-dependent activation of NRF2 promotes an invasive phenotype in BRAF mutant melanoma cells. Mol. Carcinog. 60: 331-341.
5. Zhang, S., et al. 2022. Small-molecule NSC59984 induces mutant p53 degradation through a ROS-ERK2-MDM2 axis in cancer cells. Mol. Cancer Res. 20: 622-636.

RESEARCH USE

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