SANTA CRUZ BIOTECHNOLOGY, INC.

Raf-1 siRNA (h): sc-29462

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.

CHROMOSOMAL LOCATION

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

PRODUCT

Raf-1 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-1 shRNA Plasmid (h): sc-29462-SH and Raf-1 shRNA (h) Lentiviral Particles: sc-29462-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-1 siRNA (h) is recommended for the inhibition of Raf-1 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-1 (E-10): sc-7267 is recommended as a control antibody for monitoring of Raf-1 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 Raf-1 gene expression knockdown using RT-PCR Primer: Raf-1 (h)-PR: sc-29462-PR (20 µl, 494 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Kunnimalaiyaan, M., et al. 2007. Neuroendocrine tumor cell growth inhibition by ZM336372 through alterations in multiple signaling pathways. Surgery 142: 959-964.
2. Liu, H., et al. 2011. Hepatitis B virus large surface antigen promotes liver carcinogenesis by activating the Src/PI3K/Akt pathway. Cancer Res. 71: 7547-7557.
3. Davis, R., et al. 2012. TNF-α-mediated proliferation of vascular smooth muscle cells involves Raf-1-mediated inactivation of Rb and transcription of E2F1-regulated genes. Cell Cycle 11: 109-118.
4. Tang, C.H. and Tsai, C.C. 2012. CCL2 increases MMP-9 expression and cell motility in human chondrosarcoma cells via the Ras/Raf/MEK/ERK/NFκB signaling pathway. Biochem. Pharmacol. 83: 335-344.
5. Kim, Y.K., et al. 2012. Differential sensitivity of melanoma cell lines with differing B-Raf mutational status to the new oncogenic B-Raf kinase inhibitor UI-152. Cancer Lett. 320: 215-224.
6. Martel, G., et al. 2013. The protein kinase TPL2 is essential for ERK1/ERK2 activation and cytokine gene expression in airway epithelial cells exposed to pathogen-associated molecular patterns (PAMPs). PLoS ONE 8: e59116.
7. Feng, R., et al. 2017. MAPK and Hippo signaling pathways crosstalk via the Raf-1/MST-2 interaction in malignant melanoma. Oncol. Rep. 38: 1199-1205.
8. Kizilboga, T., et al. 2019. Bag-1 stimulates Bad phosphorylation through activation of Akt and Raf kinases to mediate cell survival in breast cancer. BMC Cancer 19: 1254.
9. 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.

PROTOCOLS

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