

κB-Ras1 siRNA (h): sc-41796

BACKGROUND

Small guanosine triphosphatases, typified by the mammalian Ras proteins, play major roles in the regulation of numerous cellular pathways. IκB-interacting Ras-like proteins, κB-Ras1 and κB-Ras2, belong to a subclass of evolutionarily conserved Ras-like proteins that differ from other Ras proteins in containing amino acids at positions 12 and 61 that are similar to those present in the oncogenic forms of Ras. These Ras-like proteins, κB-Ras1 and κB-Ras2, interact with the PEST domains of IκBα and IκBβ and decrease their rate of degradation. κB-Ras2 shows 71% identity to κB-Ras1. In cells, κB-Ras proteins are associated only with NFκB:IκBβ complexes and therefore may provide an explanation for the slower rate of degradation of IκBβ compared with IκBα.

REFERENCES

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3. Bos, J.L. 1998. All in the family? New insights and questions regarding interconnectivity of Ras, Rap1 and Ral. *EMBO J.* 17: 6776-6782.
4. May, M.J., et al. 1998. Signal transduction through NFκB. *Immunol. Today* 19: 80-88.
5. Bos, J.L. 1998. The Ras gene family and human carcinogenesis. *Mutat. Res.* 195: 255-271.
6. Fenwick, C., et al. 2000. A subclass of Ras proteins that regulate the degradation of IκB. *Science* 287: 869-873.
7. Chen, Y., et al. 2003. κB-Ras binds to the unique insert within the ankyrin repeat domain of IκBβ and regulates cytoplasmic retention of IκBβ x NFκB complexes. *J. Biol. Chem.* 278: 23101-23106.
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CHROMOSOMAL LOCATION

Genetic locus: NKIRAS1 (human) mapping to 3p24.2.

PRODUCT

κB-Ras1 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 μM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see κB-Ras1 shRNA Plasmid (h): sc-41796-SH and κB-Ras1 shRNA (h) Lentiviral Particles: sc-41796-V as alternate gene silencing products.

For independent verification of κB-Ras1 (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-41796A, sc-41796B and sc-41796C.

RESEARCH USE

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

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

κB-Ras1 siRNA (h) is recommended for the inhibition of κB-Ras1 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

κB-Ras1 (E-3): sc-271169 is recommended as a control antibody for monitoring of κB-Ras1 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 κB-Ras1 gene expression knockdown using RT-PCR Primer: κB-Ras1 (h)-PR: sc-41796-PR (20 μl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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