SANTA CRUZ BIOTECHNOLOGY, INC.

κB-Ras1 siRNA (m): sc-41797

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. κB-Ras1 and κB-Ras2 interact with the PEST domains of IκBα and IκBβ and decrease their rate of degradation. κB-Ras2 has a molecular mass of 22 kDa and 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. and Ghosh, S. 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·NFκB complexes. J. Biol. Chem. 278:23101-23106.
8. Chen, Y., et al. 2004. Inhibition of NFκB activity by IκBβ in association with κB-Ras. Mol. Cell. Biol. 24: 3048-3056.

CHROMOSOMAL LOCATION

Genetic locus: Nkiras1 (mouse) mapping to 14 A2.

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. Suit-able 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 (m) is recommended for the inhibition of κB-Ras1 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

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 (m)-PR: sc-41797-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.