

H-Ras siRNA (m): sc-29341

BACKGROUND

The mammalian Ras (also designated v-Ha-Ras, Harvey rat sarcoma viral oncogene homolog, HRAS1, K-Ras, N-Ras, RASH1 or c-Bas/Has) gene family consists of the Harvey and Kirsten Ras genes (c-H-Ras1 and c-K-Ras2), an inactive pseudogene of each (c-H-Ras2 and c-K-Ras1) and the N-Ras gene. The three Ras oncogenes, H-Ras, K-Ras and N-Ras, encode proteins with GTP/GDP binding and GTPase activity. Ras proteins alternate between an inactive form bound to GDP and an active form bound to GTP, activated by a guanine nucleotide-exchange factor (GEF) and inactivated by a GTPase-activating protein (GAP). Ras nomenclature originates from the characterization of human DNA sequences homologous to cloned DNA fragments containing oncogenic sequences of a type C mammalian retrovirus, the Harvey strain of murine sarcoma virus (HaMSV), derived from the rat. Under normal conditions, Ras family members influence cell growth and differentiation events in a sub-cellular membrane compartmentalization-based signaling system. Oncogenic Ras can deregulate processes that control both cell proliferation and apoptosis. The Ras superfamily of GTP hydrolysis-coupled signal transduction relay proteins can be subclassified into Ras, Rho, Rab and ARF families.

REFERENCES

1. Wong-Staal, F., et al. 1981. Three distinct genes in human DNA related to the transforming genes of mammalian sarcoma retroviruses. *Science* 213: 226-228.
2. Cox, A.D. and Der, C.J. 2003. The dark side of Ras: regulation of apoptosis. *Oncogene* 22: 8999-9006.
3. Colicelli, J. 2004. Human Ras superfamily proteins and related GTPases. *Sci. STKE* 2004: RE13.

CHROMOSOMAL LOCATION

Genetic locus: Hras (mouse) mapping to 7 F5.

PRODUCT

H-Ras siRNA (m) 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 H-Ras shRNA Plasmid (m): sc-29341-SH and H-Ras shRNA (m) Lentiviral Particles: sc-29341-V as alternate gene silencing products.

For independent verification of H-Ras (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-29341A, sc-29341B and sc-29341C.

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

H-Ras siRNA (m) is recommended for the inhibition of H-Ras 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 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

H-Ras (M90): sc-53959 is recommended as a control antibody for monitoring of H-Ras 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 H-Ras gene expression knockdown using RT-PCR Primer: H-Ras (m)-PR: sc-29341-PR (20 μ l, 410 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Contente, S., et al. 2011. H-Ras localizes to cell nuclei and varies with the cell cycle. *Genes Cancer* 2: 166-172.
2. Wang, Q., et al. 2011. Focal adhesions and Ras are functionally and spatially integrated to mediate IL-1 activation of ERK. *FASEB J.* 25: 3448-3464.

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.