



E-Ras siRNA (m): sc-60565

BACKGROUND

The Ras-encoded family of proteins bind to GDP and to GTP with high affinity. They possess a low level of intrinsic GTPase activity that can be stimulated more than 100-fold by interaction with cytosolic GTPase activating protein (GAP). Ras family members include H-Ras, K-Ras, N-Ras, M-Ras, R-Ras, E-Ras, Rheb, TC 21, RASL11B and Rad GTPase. H-Ras and K-Ras were first identified as oncogenes of acutely transforming RNA tumor viruses. Subsequently, mutated Ras genes have been found in many human tumors, providing evidence for a common genetic target in cancer. In mammals, a variety of extracellular growth factors that act through protein tyrosine kinase receptors, such as Insulin, platelet-derived growth factor and nerve growth factor, require Ras to exert their effects. Embryonic stem cell-expressed Ras (E-Ras) is a 277 amino acid protein that localizes to the cytoplasmic membrane and shares 43%, 46% and 47% identity with H-Ras, K-Ras and N-Ras, respectively. E-Ras contains five highly conserved domains essential for small G proteins and a CAAX motif.

REFERENCES

1. Miyoshi, J., et al. 1984. The human c-Ha-Ras2 is a processed pseudogene inactivated by numerous base substitutions. *Nucleic Acids Res.* 12: 1821-1828.
2. Bauer, P.L., et al. 2002. Anticancer action of 4-iodo-3-nitrobenzamide in combination with buthionine sulfoximine: inactivation of poly(ADP-ribose) polymerase and tumor glycolysis and the appearance of a poly(ADP-ribose) polymerase protease. *Biochem. Pharmacol.* 63: 455-462.

CHROMOSOMAL LOCATION

Genetic locus: Eras (mouse) mapping to X A1.1.

PRODUCT

E-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 E-Ras shRNA Plasmid (m): sc-60565-SH and E-Ras shRNA (m) Lentiviral Particles: sc-60565-V as alternate gene silencing products.

For independent verification of E-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-60565A, sc-60565B and sc-60565C.

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

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

E-Ras (B-12): sc-393268 is recommended as a control antibody for monitoring of E-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 E-Ras gene expression knockdown using RT-PCR Primer: E-Ras (m)-PR: sc-60565-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Chen, L. and Khillan, J.S. 2010. A novel signaling by Vitamin A/retinol promotes self renewal of mouse embryonic stem cells by activating PI3K/Akt signaling pathway via Insulin-like growth factor-1 receptor. *Stem Cells* 28: 57-63.

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.