

NRAGE siRNA (h): sc-37320

BACKGROUND

The neurotrophin family of growth factors (NGF) function to regulate neuronal differentiation, synaptic activity and neuronal survival, as well as axonal and dendritic growth. The melanoma-associated antigen (MAGE) family consists of a number of antigens recognized by cytotoxic T lymphocytes. Neurotrophin receptor-interacting MAGE homolog (NRAGE) binds the p75 neurotrophin receptor and associates with the plasma membrane when NGF binds p75NTR. The critical factors for NRAGE association lie within the juxtamembrane domain of p75NTR. Overexpression of NRAGE stimulates cell cycle arrest and allows NGF-dependent apoptosis within sympathetic neuron precursors cells. NRAGE is expressed in the medulla oblongata during development and motor-neurons. Structural similarities suggest NRAGE, and the MAGE protein necdin, (Ndn), mediate cell cycle effects through a shared mechanism.

REFERENCES

1. Farinas, I. 1999. Neurotrophin actions during the development of the peripheral nervous system. *Microsc. Res. Tech.* 45: 233-242.
2. Okami, J., et al. 2000. Genetic detection for micrometastasis in lymph node of biliary tract carcinoma. *Clin. Cancer Res.* 6: 2326-2332.
3. Granelli, P., et al. 2000. Melanoma antigen genes 1 and 2 are differentially expressed in human gastric and cardiac carcinomas. *Scand. J. Gastroenterol.* 35: 528-533.
4. Klein, C., et al. 2000. Comparative analysis of genetically modified dendritic cells and tumor cells as therapeutic cancer vaccines. *J. Exp. Med.* 191: 1699-1708.
5. Busam, K.J., et al. 2000. Immunoreactivity with the anti-MAGE antibody 57B in malignant melanoma: frequency of expression and correlation with prognostic parameters. *Mod. Pathol.* 13: 459-465.
6. Kobayashi, Y., et al. 2000. Expression of MAGE, GAGE and BAGE genes in human liver diseases: utility as molecular markers for hepatocellular carcinoma. *J. Hepatol.* 32: 612-617.
7. Salehi, A.H., et al. 2000. NRAGE, a novel MAGE protein, interacts with the p75 neurotrophin receptor and facilitates nerve growth factor-dependent apoptosis. *Neuron* 27: 279-288.

CHROMOSOMAL LOCATION

Genetic locus: MAGED1 (human) mapping to Xp11.22.

PRODUCT

NRAGE 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NRAGE shRNA Plasmid (h): sc-37320-SH and NRAGE shRNA (h) Lentiviral Particles: sc-37320-V as alternate gene silencing products.

For independent verification of NRAGE (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-37320A, sc-37320B and sc-37320C.

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

NRAGE siRNA (h) is recommended for the inhibition of NRAGE 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 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

NRAGE (F-9): sc-393291 is recommended as a control antibody for monitoring of NRAGE 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 NRAGE gene expression knockdown using RT-PCR Primer: NRAGE (h)-PR: sc-37320-PR (20 μ l, 575 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.