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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

NRAGE shRNA (m) Lentiviral Particles: sc-45975-V

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. McAllister, A.K., et al. 1999. Neurotrophins and synaptic plasticity. *Annu. Rev. Neurosci.* 22: 295-318.
3. Okami, J., et al. 2000. Genetic detection for micrometastasis in lymph node of biliary tract carcinoma. *Clin. Cancer Res.* 6: 2326-2332.
4. 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.
5. 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.

CHROMOSOMAL LOCATION

Genetic locus: Maged1 (mouse) mapping to X C3.

PRODUCT

NRAGE shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see NRAGE siRNA (m): sc-45975 and NRAGE shRNA Plasmid (m): sc-45975-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

NRAGE shRNA (m) Lentiviral Particles is recommended for the inhibition of NRAGE expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

NRAGE (H-7): sc-398493 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NRAGE gene expression knockdown using RT-PCR Primer: NRAGE (m)-PR: sc-45975-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.