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NuMA shRNA (m) Lentiviral Particles: sc-44345-V

BACKGROUND

There are a multitude of structural components in the nucleus that sustain proper structure and function relationships with respect to nuclear assembly and mitosis. The human nuclear mitotic apparatus protein gene, also designated NuMA, maps to chromosome 11q13 and encodes a noncentrosomal protein. NuMA possesses microtubule (MT) binding capacity via its carboxyl terminal region and is involved in spindle pole organization. NuMA is essential for the organization and stabilization of spindle poles from early mitosis until at least the onset of anaphase. During interphase, NuMA is present throughout the nucleus and upon entering mitosis, localizes to the spindle apparatus. During mitosis, NuMA forms aggregates that interact with microtubules and certain motor proteins and as a result may draw together the minus-ends of microtubules, thereby helping to organize them into a bipolar spindle. In contrast to mitotic cells, post-mitotic neurons display NuMA both in the nucleus and in the cytoplasm. Elevated levels of NuMA expression have been reported in cancer patients, particularly in colorectal carcinoma and early colorectal cancers.

REFERENCES

- Lydersen, B.K., et al. 1980. Human-specific nuclear protein that associates with the polar region of the mitotic apparatus: distribution in a human/hamster hybrid cell. *Cell* 22: 489-499.
- Sparks, C.A., et al. 1993. Assignment of the nuclear mitotic apparatus protein NuMA gene to human chromosome 11q13. *Genomics* 17: 222-224.
- Ferhat, L., et al. 1998. The nuclear/mitotic apparatus protein NuMA is a component of the somatodendritic microtubule arrays of the neuron. *J. Neurocytol.* 27: 887-899.
- Hasholzner, U., et al. 1999. Nuclear mitotic apparatus protein (NuMA) in benign and malignant diseases. *Anticancer Res.* 19: 2415-2420.

CHROMOSOMAL LOCATION

Genetic locus: *Numa1* (mouse) mapping to 7 E3.

PRODUCT

NuMA 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 NuMA siRNA (m): sc-44345 and NuMA shRNA Plasmid (m): sc-44345-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

NuMA shRNA (m) Lentiviral Particles is recommended for the inhibition of NuMA 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

NuMA (H-300): sc-48773 is recommended as a control antibody for monitoring of NuMA 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NuMA gene expression knockdown using RT-PCR Primer: NuMA (m)-PR: sc-44345-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.