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NDR1 shRNA (m) Lentiviral Particles: sc-44367-V

BACKGROUND

The nuclear Dbf2-related kinases (NDR1 and NDR2) participate in the regulation of cell division and morphology and may be implicated in tumor progression. NDR1 and NDR2 share 86% amino acid identity, but differ in their expression pattern. NDR1 localizes to the nucleus, while NDR2 exhibits punctate cytoplasmic distribution. Also, NDR1 expression appears highest in spleen, lung and thymus, whereas NDR2 shows highest expression in the gastrointestinal tract. However, both NDR1 and NDR2 are regulated by phosphorylation and by the Ca²⁺-binding protein S-100B. NDR1 and NDR2 may also play a role in the HIV-1 life cycle. Both proteins are cleaved by the HIV-1 protease (PR), which inhibits their enzymatic activity and alters the subcellular localization of NDR2. The genes encoding human NDR1 and NDR2 map to chromosomes 6p21 and 12p11.23, respectively.

REFERENCES

1. Tamaskovic, R., et al. 2003. Mechanism of Ca²⁺-mediated regulation of NDR protein kinase through autophosphorylation and phosphorylation by an upstream kinase. *J. Biol. Chem.* 278: 6710-6718.
2. Bichsel, S.J., et al. 2004. Mechanism of activation of NDR (nuclear Dbf2-related) protein kinase by the hMOB1 protein. *J. Biol. Chem.* 279: 35228-35235.
3. Devroe, E., et al. 2004. Human Mob proteins regulate the NDR1 and NDR2 serine-threonine kinases. *J. Biol. Chem.* 279: 24444-24451.
4. Stegert, M.R., et al. 2004. Regulation of NDR2 protein kinase by multi-site phosphorylation and the S100B calcium-binding protein. *J. Biol. Chem.* 279: 23806-23812.
5. Devroe, E., et al. 2005. HIV-1 incorporates and proteolytically processes human NDR1 and NDR2 serine-threonine kinases. *Virology* 331: 181-189.

CHROMOSOMAL LOCATION

Genetic locus: Stk38 (mouse) mapping to 17 A3.3.

PRODUCT

NDR1 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 µl frozen stock containing 1.0 x 10⁶ 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 NDR1 siRNA (m): sc-44367 and NDR1 shRNA Plasmid (m): sc-44367-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

NDR1 shRNA (m) Lentiviral Particles is recommended for the inhibition of NDR1 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ 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

NDR1 (A-8): sc-365555 is recommended as a control antibody for monitoring of NDR1 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 NDR1 gene expression knockdown using RT-PCR Primer: NDR1 (m)-PR: sc-44367-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.