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NDR1 siRNA (h): sc-44366

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 6p21.31 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. Devroe, E., et al. 2004. Human Mob proteins regulate the NDR1 and NDR2 serine-threonine kinases. *J. Biol. Chem.* 279: 24444-24451.
3. 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.
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 (human) mapping to 6p21.31.

PRODUCT

NDR1 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 NDR1 shRNA Plasmid (h): sc-44366-SH and NDR1 shRNA (h) Lentiviral Particles: sc-44366-V as alternate gene silencing products.

For independent verification of NDR1 (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-44366A, sc-44366B and sc-44366C.

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.

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

NDR1 siRNA (h) is recommended for the inhibition of NDR1 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

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 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 NDR1 gene expression knockdown using RT-PCR Primer: NDR1 (h)-PR: sc-44366-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.