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NDR-2 siRNA (m): sc-45829

BACKGROUND

The nuclear Dbf2-related kinases (NDR-1 and NDR-2) participate in the regulation of cell division and morphology and may be implicated in tumor progression. NDR-1 and NDR-2 share 86% amino acid identity, but differ in their expression pattern. NDR-1 localizes to the nucleus, while NDR-2 exhibits punctate cytoplasmic distribution. Also, NDR-1 expression appears highest in spleen, lung and thymus, whereas NDR-2 shows highest expression in the gastrointestinal tract. However, both NDR-1 and NDR-2 are regulated by phosphorylation and by the Ca²⁺-binding protein S100B. NDR-1 and NDR-2 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 NDR-2. The genes encoding human NDR-1 and NDR-2 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. Stegert, M.R., et al. 2004. Regulation of NDR-2 protein kinase by multi-site phosphorylation and the S100B calcium-binding protein. *J. Biol. Chem.* 279: 23806-23812.
3. Devroe, E., et al. 2004. Human Mob proteins regulate the NDR-1 and NDR-2 serine-threonine kinases. *J. Biol. Chem.* 279: 24444-24451.
4. 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.
5. Devroe, E., et al. 2005. HIV-1 incorporates and proteolytically processes human NDR-1 and NDR-2 serine-threonine kinases. *Virology* 331: 181-189.

CHROMOSOMAL LOCATION

Genetic locus: Stk38l (mouse) mapping to 6 G3.

PRODUCT

NDR2 siRNA (m) 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 NDR2 shRNA Plasmid (m): sc-45829-SH and NDR2 shRNA (m) Lentiviral Particles: sc-45829-V as alternate gene silencing products.

For independent verification of NDR2 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-45829A, sc-45829B and sc-45829C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com or our catalog 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

NDR2 siRNA (m) is recommended for the inhibition of NDR2 expression in mouse 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

NDR1/2 (E-2): sc-271703 is recommended as a control antibody for monitoring of NDR2 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 NDR2 gene expression knockdown using RT-PCR Primer: NDR2 (m)-PR: sc-45829-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.