

Produktinformation



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Id1 shRNA (h2) Lentiviral Particles: sc-44267-V



The Power to Question

BACKGROUND

Members of the ld family of basic helix-loop-helix (bHLH) proteins include ld1, ld2, ld3 and ld4. They are ubiquitously expressed and dimerize with members of the class A and B HLH proteins. Due to the absence of the basic region, the resulting heterodimers cannot bind DNA. The ld-type proteins thus appear to negatively regulate DNA binding of bHLH proteins. Since ld1 inhibits DNA binding of E12 and Myo D, it apparently functions to inhibit muscle-specific gene expression. Under conditions that facilitate muscle cell differentiation, the ld protein levels fall, allowing E12 and/or E47 to form heterodimers with Myo D and myogenin, which in turn activate myogenic differentiation. It has been shown that expression of each of the ld proteins is strongly dependent on growth factor activation and that reduction of ld mRNA levels by antisense oligonucleotides leads to a delayed reentry of arrested cells into the cell cycle following growth factor stimulation.

REFERENCES

- Benezra, R., et al. 1990. The protein Id: a negative regulator of helix-loophelix DNA binding proteins. Cell 61: 49-59.
- Christy, B.A., et al. 1991. An Id-related helix-loop-helix protein encoded by a growth factor-inducible gene. Proc. Natl. Acad. Sci. USA 88: 1815-1819.
- 3. Sun, X., et al. 1991. Id proteins Id1 and Id2 selectively inhibit DNA binding by one class of helix-loop-helix proteins. Mol. Cell. Biol. 11: 5603-5611.
- Neuhold, L.A. and Wold, B. 1993. HLH forced dimers: tethering MyoD to E47 generates a dominant positive myogenic factor insulated from negative regulation by Id. Cell 74: 1033-1042.
- Riechmann, V., et al. 1994. The expression pattern of Id4, a novel dominant negative helix-loop-helix protein, is distinct from Id1, Id2 and Id3. Nucl. Acids Res. 22: 749-755.

CHROMOSOMAL LOCATION

Genetic locus: ID1 (human) mapping to 20q11.21.

PRODUCT

ld1 shRNA (h2) 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 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 ld1 siRNA (h2): sc-44267 and ld1 shRNA Plasmid (h2): sc-44267-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

ld1 shRNA (h2) Lentiviral Particles is recommended for the inhibition of ld1 expression in human cells.

SUPPORT REAGENTS

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

ld1 (F-10): sc-365654 is recommended as a control antibody for monitoring of ld1 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) Immunofluo-rescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Id1 gene expression knockdown using RT-PCR Primer: Id1 (h2)-PR: sc-44267-PR (20 μ I). 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.

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