



## Id3 siRNA (h2): sc-63297

### BACKGROUND

Members of the Id family of basic helix-loop-helix (bHLH) proteins include Id1, Id2, Id3 and Id4. 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 Id-type proteins thus appear to negatively regulate DNA binding of bHLH proteins. Since Id1 inhibits DNA binding of E12 and MyoD, it apparently functions to inhibit muscle-specific gene expression. Under conditions that facilitate muscle cell differentiation, the Id protein levels fall, allowing E12 and/or E47 to form heterodimers with MyoD and myogenin, which in turn activate myogenic differentiation. It has been shown that expression of each of the Id proteins is strongly dependent on growth factor activation and that reduction of Id mRNA levels by antisense oligonucleotides leads to a delayed reentry of arrested cells into the cell cycle following growth factor stimulation.

### REFERENCES

1. Benezra, R., et al. 1990. The protein Id: a negative regulator of helix-loop-helix DNA binding proteins. *Cell* 61: 49-59.
2. 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.
4. Neuhold, L.A., et al. 1993. HLH forced dimers: tethering MyoD to E47 generates a dominant positive myogenic factor insulated from negative regulation by Id. *Cell* 74: 1033-1042.
5. 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. *Nucleic Acids Res.* 22: 749-755.
6. Barone, M.V., et al. 1994. Id proteins control growth induction in mammalian cells. *Proc. Natl. Acad. Sci. USA* 91: 4985-4988.
7. Hara, E., et al. 1994. Id-related genes encoding helix-loop-helix proteins are required for G<sub>1</sub> progression and are repressed in senescent human fibroblasts. *J. Biol. Chem.* 269: 2139-2145.

### CHROMOSOMAL LOCATION

Genetic locus: ID3 (human) mapping to 1p36.12; Id3 (mouse) mapping to 4 D3.

### PRODUCT

Id3 siRNA (h2) is a pool of 3 target-specific 20-25 nt siRNAs designed to knock down gene expression. Each vial contains 3 nmol of lyophilized siRNA, sufficient for a 10  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections.

For independent verification of Id3 (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3 nmol of lyophilized siRNA. These include: sc-63297A, sc-63297B and sc-63297C.

### RESEARCH USE

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

### 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

### APPLICATIONS

Id3 siRNA (h2) is recommended for the inhibition of Id3 expression in human cells.

Id3 (C-20): sc-490 is recommended as a control antibody for Western Blotting (starting dilution 1:100, dilution range 1:100-1:1,000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) protein detection using the recommended secondary reagents listed below.

### RECOMMENDED SECONDARY REAGENTS

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.

### 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  $\mu$ M in 60  $\mu$ 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. Semi-quantitative RT-PCR may be performed using RT-PCR Primer: Id3 (h2)-PR: sc-63297-PR (20  $\mu$ l).

### PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog for detailed protocols and support products.