

# HRT3 siRNA (h): sc-37918

## BACKGROUND

The LIN-12/Notch family of transmembrane receptors plays a central role in development by regulating cell fate and establishing boundaries of gene expression. Notch signaling activates the Hairy/Enhancer of split [H/E(spl)] genes, which encode basic helix-loop-helix (bHLH) transcriptional repressors that are critical for directing embryonic patterning and development. The Hairy-related transcription factors (HRTs) comprise a subclass of bHLH proteins that exhibit structural similarity with the H/E(spl) proteins and include HRT1, HRT2 and HRT3. The HRT family (also designated Hesr, Hey, CHF and Gridlock) contain a bHLH domain, an Orange domain and a novel YRPW domain, which is absent in HRT3. The Hairy-related genes map to human chromosomes 8q21, 6q21 and 1p34.3 for HRT1, HRT2 and HRT3, respectively, and are downstream targets for Notch signaling. HRT1 is expressed in the somitic mesoderm, central nervous system, kidney, heart, nasal epithelium and limb buds in murine embryos as well as in adult tissues. It has altered expression in many breast, lung and kidney tumors. Like HRT1, HRT2 and HRT3 are also expressed in developing somites, heart and nervous system.

## REFERENCES

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2. Kokubo, H., et al. 1999. Identification and expression of a novel family of bHLH cDNAs related to *Drosophila* Hairy and Enhancer of split. *Biochem. Biophys. Res. Commun.* 260: 459-465.
3. Nakagawa, O., et al. 1999. HRT1, HRT2, and HRT3: a new subclass of bHLH transcription factors marking specific cardiac, somitic, and pharyngeal arch segments. *Dev. Biol.* 216: 72-84.
4. Leimeister, C., et al. 1999. Hey genes: a novel subfamily of Hairy and Enhancer of split related genes specifically expressed during mouse embryogenesis. *Mech. Dev.* 85: 173-177.
5. Leimeister, C., et al. 2000. Analysis of HeyL expression in wildtype and Notch pathway mutant mouse embryos. *Mech. Dev.* 98: 175-178.
6. Nakagawa, O., et al. 2000. Members of the HRT family of basic helix-loop-helix proteins act as transcriptional repressors downstream of Notch signaling. *Proc. Natl. Acad. Sci. USA* 97: 13655-13660.
7. Steidl, C., et al. 2000. Characterization of the human and mouse HEY1, HEY2, and HEYL genes: cloning, mapping, and mutation screening of a new bHLH gene family. *Genomics* 66: 195-203.
8. Henderson, A.M., et al. 2001. The basic helix-loop-helix transcription factor HESR1 regulates endothelial cell tube formation. *J. Biol. Chem.* 276: 6169-6176.

## CHROMOSOMAL LOCATION

Genetic locus: HEYL (human) mapping to 1p34.3.

## PROTOCOLS

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

## PRODUCT

HRT3 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see HRT3 shRNA Plasmid (h): sc-37918-SH and HRT3 shRNA (h) Lentiviral Particles: sc-37918-V as alternate gene silencing products.

For independent verification of HRT3 (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-37918A, sc-37918B and sc-37918C.

## 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

HRT3 siRNA (h) is recommended for the inhibition of HRT3 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  $\mu$ M in 66  $\mu$ 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.

## GENE EXPRESSION MONITORING

HRT3 (3555C3a): sc-81294 is recommended as a control antibody for monitoring of HRT3 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).

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor HRT3 gene expression knockdown using RT-PCR Primer: HRT3 (h)-PR: sc-37918-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## RESEARCH USE

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