

Hrs siRNA (h2): sc-270003

BACKGROUND

The hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) is a zinc-finger protein that interacts with STAM and undergoes tyrosine phosphorylation in response to IL2, CSF2 or HGF. Hrs is involved in intracellular trafficking and signal transduction and is associated with early endosomes. Hrs contains a phosphatidylinositol 3-phosphate-binding domain that contributes to its endosomal targeting, where Hrs co-localizes with Clathrin via a Clathrin box motif at the carboxy-terminus of Hrs. Hrs is essential for ventral folding morphogenesis and shares structural similarity to the yeast protein Vps27p, which is involved in vacuolar protein sorting. The human Hrs gene, which maps to chromosome 17q25.3, encodes a 777 amino acid protein. In Schwann cells, Hrs co-localizes at endosomes with the tumor suppressor protein schwannomin, suggesting a role for schwannomin in Hrs-mediated cell signaling.

REFERENCES

1. Asao, H., et al. 1997. Hrs is associated with STAM, a signal-transducing adaptor molecule. Its suppressive effect on cytokine-induced cell growth. *J. Biol. Chem.* 272: 32785-32791.
2. Lu, L., et al. 1998. Human Hrs, a tyrosine kinase substrate in growth factor-stimulated cells: cDNA cloning and mapping of the gene to chromosome 17. *Gene* 213: 125-132.
3. Scoles, D.R., et al. 2000. The neurofibromatosis 2 tumor suppressor protein interacts with hepatocyte growth factor-regulated tyrosine kinase substrate. *Hum. Mol. Genet.* 9: 1567-1574.
4. Raiborg, C., et al. 2001. Hrs recruits clathrin to early endosomes. *EMBO J.* 20: 5008-5021.
5. Raiborg, C., et al. 2001. Function of Hrs in endocytic trafficking and signalling. *Biochem. Soc. Trans.* 29: 472-475.
6. LocusLink Report (Locus ID: 9146) <http://www.ncbi.nlm.nih.gov/LocusLink/>

CHROMOSOMAL LOCATION

Genetic locus: HGS (human) mapping to 17q25.3.

PRODUCT

Hrs siRNA (h2) 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 Hrs shRNA Plasmid (h2): sc-270003-SH and Hrs shRNA (h2) Lentiviral Particles: sc-270003-V as alternate gene silencing products.

For independent verification of Hrs (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270003A, sc-270003B and sc-270003C.

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

Hrs siRNA (h2) is recommended for the inhibition of Hrs 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

Hrs (C-7): sc-271455 is recommended as a control antibody for monitoring of Hrs 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 Hrs gene expression knockdown using RT-PCR Primer: Hrs (h2)-PR: sc-270003-PR (20 μ 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.