

LASS1 siRNA (h): sc-62543

BACKGROUND

The LASS (longevity assurance homolog) family members are highly conserved from yeasts to mammals. Six members of this family of proteins involved in sphingolipid synthesis have been characterized (LASS1, LASS2, LASS3, LASS4, LASS5 and LASS6). LASS1, also called LAG1, is a 350 amino acid ceramide synthase located in the membrane of the endoplasmic reticulum. The gene coding LASS1 is bicistronic, containing both the LASS1 and GDF1 open reading frames. Two isoforms of LASS1 have been characterized. Isoform 2 lacks the last 13 amino acids of the intact protein (isoform 1). The cell death and growth inhibition in head and neck squamous cell carcinoma (HNSCC) brought on by the chemotherapeutic agents gemcitabine and doxorubicin via the activation of caspase-3 and caspase-9 may involve LASS1 overexpression.

REFERENCES

1. Venkataraman, K., et al. 2002. Upstream of growth and differentiation factor 1 (uog1), a mammalian homolog of the yeast longevity assurance gene 1 (LAG1), regulates N-stearoyl-sphinganine (C18-(dihydro)ceramide) synthesis in a fumonisins B1-independent manner in mammalian cells. *J. Biol. Chem.* 277: 35642-35649.
2. Riebeling, C., et al. 2003. Two mammalian longevity assurance gene (LAG1) family members, trh1 and trh4, regulate dihydroceramide synthesis using different fatty acyl-CoA donors. *J. Biol. Chem.* 278: 43452-43459.
3. Mizutani, Y., et al. 2006. LASS3 (longevity assurance homologue 3) is a mainly testis-specific (dihydro)ceramide synthase with relatively broad substrate specificity. *Biochem. J.* 398: 531-538.
4. Wang, B., et al. 2007. Cloning and characterization of a LASS1-GDF1 transcript in rat cerebral cortex: conservation of a bicistronic structure. *DNA Seq.* 18: 92-103.
5. Senkal, C.E., et al. 2007. Role of human longevity assurance gene 1 and C18-ceramide in chemotherapy-induced cell death in human head and neck squamous cell carcinomas. *Mol. Cancer Ther.* 6: 712-722.

CHROMOSOMAL LOCATION

Genetic locus: CERS1 (human) mapping to 19p13.11.

PRODUCT

LASS1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see LASS1 shRNA Plasmid (h): sc-62543-SH and LASS1 shRNA (h) Lentiviral Particles: sc-62543-V as alternate gene silencing products.

For independent verification of LASS1 (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-62543A, sc-62543B and sc-62543C.

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

LASS1 siRNA (h) is recommended for the inhibition of LASS1 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

LASS1 (3F9): sc-293497 is recommended as a control antibody for monitoring of LASS1 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 LASS1 gene expression knockdown using RT-PCR Primer: LASS1 (h)-PR: sc-62543-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.