

LAGE-1 siRNA (h): sc-60916

BACKGROUND

Cancer-testis (CT) antigens are expressed in various cancers, and absent in normal adult tissues, thereby functioning as useful vaccine targets for immunotherapy. The LAGE-1 gene gives rise to two CT products: a 180 amino acid protein containing two glycine-rich regions and a hydrophobic stretch near the C-terminus, and an alternate 210 amino acid protein that lacks the hydrophobic stretch near the C terminus. Both proteins are expressed in melanoma cell lines, lung tumors, sarcomas, and other types of cancer cells, but not in normal tissues except for testis and placenta. LAGE-1 activates type-1 melanoma-reactive CD4⁺ helper-T cells and, thus, promotes long-lasting antitumor responses.

REFERENCES

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3. Usener, D., et al. 2003. Seroreactivity against MAGE-A and LAGE-1 proteins in melanoma patients. *Br. J. Dermatol.* 149: 282-288.
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5. Xing, B.C., et al. 2004. Expression of NY-ESO-1/LAGE-1 genes in hepatocellular carcinoma and autologous humoral responses induced thereby. *Zhonghua Yi Xue Za Zhi* 84: 1980-1982.
6. Bolli, M., et al. 2005. NY-ESO-1/LAGE-1 coexpression with MAGE-A cancer/testis antigens: a tissue microarray study. *Int. J. Cancer* 115: 960-966.
7. Sato, S., et al. 2005. Quantitative real-time RT-PCR analysis of NY-ESO-1 and LAGE-1a mRNA expression in normal tissues and tumors, and correlation of the protein expression with the mRNA copy number. *Int. J. Oncol.* 26: 57-63.
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CHROMOSOMAL LOCATION

Genetic locus: CTAG2 (human) mapping to Xq28.

PRODUCT

LAGE-1 siRNA (h) is a pool of 2 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 LAGE-1 shRNA Plasmid (h): sc-60916-SH and LAGE-1 shRNA (h) Lentiviral Particles: sc-60916-V as alternate gene silencing products.

For independent verification of LAGE-1 (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-60916A and sc-60916B.

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

LAGE-1 siRNA (h) is recommended for the inhibition of LAGE-1 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

LAGE-1 (3H1): sc-517172 is recommended as a control antibody for monitoring of LAGE-1 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 LAGE-1 gene expression knockdown using RT-PCR Primer: LAGE-1 (h)-PR: sc-60916-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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.