SANTA CRUZ BIOTECHNOLOGY, INC.

MAGE-A4 siRNA (h): sc-45955

BACKGROUND

The melanoma-associated antigen (MAGE) family consists of a number of antigens recognized by cytotoxic T lymphocytes. The MAGE genes were initially isolated from different kinds of tumors, and based on their virtually exclusive tumor-specific expression in adult tissues, they have been used as targets for cancer immunotherapy. MAGE genes encode for tumor-rejection antigens and are expressed in tumors of different histologic types, but not in normal tissues, with the exception of testis and placenta. Malignant neoplasms have been shown to express MAGE genes, notably MAGE-A4. Expression correlates significantly with poorly differentiated tumors of cervical lineage, and while MAGE-A4 localizes to the nucleus in well-differentiated tumors, it occupies both the nucleus and cytoplasm of poorly differentiated cancer cells. Expression of MAGE-4 is not limited to cervical carcinoma; more than 50 percent of carcinomas of the esophagus, head and neck, lung, and bladder also express MAGE-A4, where it prompts cytolytic T lymphocyte targeting, suggesting it may serve as a target for antitumoral vaccination.

REFERENCES

1. Aubry, F., et al. 2001. MAGE-A4, a germ cell specific marker, is expressed differentially in testicular tumors. Cancer 92: 2778-2785.
2. Zhang, Y., et al. 2002. A MAGE-A4 peptide presented by HLA-B37 is recognized on human tumors by cytolytic T lymphocytes. Tissue Antigens 60: 365-371.
3. Yakirevich, E., et al. 2003. Morphometrical quantification of spermatogonial germ cells with the 57B anti-MAGE-A4 antibody in the evaluation of testicular biopsies for zoospermia. Appl. Immunohistochem. Mol. Morphol. 11: 37-44.
4. Sarcevic, B., et al. 2003. Expression of cancer/testis tumor associated antigens in cervical squamous cell carcinoma. Oncology 64: 443-449.
5. Rajpert-De Meyts, E., et al. 2003. The immunohistochemical expression pattern of Chk2, p53, $p19^{INK4d}$, MAGE-A4 and other selected antigens provides new evidence for the premeiotic origin of spermatocytic seminoma. Histopathology 42: 217-226.

CHROMOSOMAL LOCATION

Genetic locus: MAGEA4 (human) mapping to Xq28.

PRODUCT

MAGE-A4 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see MAGE-A4 shRNA Plasmid (h): sc-45955-SH and MAGE-A4 shRNA (h) Lentiviral Particles: sc-45955-V as alternate gene silencing products.

For independent verification of MAGE-A4 (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-45955A, sc-45955B and sc-45955C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

MAGE-A4 siRNA (h) is recommended for the inhibition of MAGE-A4 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 µM in 66 µl. 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

MAGE-A (6C1): sc-20034 is recommended as a control antibody for monitoring of MAGE-A4 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 MAGE-A4 gene expression knockdown using RT-PCR Primer: MAGE-A4 (h)-PR: sc-45955-PR (20 µl). 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.