

Melan-A siRNA (h): sc-35920

BACKGROUND

Melanoma-associated antigens recognized by cytotoxic T lymphocytes (CTL) have been grouped into three categories: melanocyte differentiation antigens, cancer/testis-specific antigens and mutated or aberrantly expressed antigens. Many of these antigens consist of peptides that are presented to T cells by HLA molecules; they represent potential targets for cancer immunotherapy. Melan-A (also designated MART-1) is a melanocyte differentiation antigen that is specific to melanomas, melanocyte cell lines and retina. Melan-A peptide is recognized by most HLA-A2-restricted tumor-specific tumor-infiltrating lymphocytes in patients with melanoma. Antimelanoma cytotoxic T lymphocytes can be generated with a Melan-A peptide, implicating Melan-A as a potential candidate for antigen-specific immunotherapy in melanoma patients.

REFERENCES

1. Chen, Y.T., et al. 1996. Serological analysis of Melan-A (MART-1), a melanocyte-specific protein homogeneously expressed in human melanomas. *Proc. Natl. Acad. Sci. USA* 93: 5915-5919.
2. Van den Eynde, B.J. and Boon, T. 1997. Tumor antigens recognized by T lymphocytes. *Int. J. Clin. Lab. Res.* 27: 81-86.
3. Kawakami, Y., et al. 1997. Production of recombinant MART-1 proteins and specific antiMART-1 polyclonal and monoclonal antibodies: use in the characterization of the human melanoma antigen MART-1. *J. Immunol. Methods* 202: 13-25.
4. Busam, K.J., et al. 1998. Expression of Melan-A (MART-1) in benign melanocytic nevi and primary cutaneous malignant melanoma. *Am. J. Surg. Pathol.* 22: 976-982.
5. Kirkin, A.F., et al. 1998. Melanoma-associated antigens recognized by cytotoxic T lymphocytes. *APMIS* 106: 665-679.
6. Loftus, D.J., et al. 1998. Peptides derived from self-proteins as partial agonists and antagonists of human CD8⁺ T-cell clones reactive to melanoma/melanocyte epitope MART1 (27-35). *Cancer Res.* 58: 2433-2439.

CHROMOSOMAL LOCATION

Genetic locus: MLANA (human) mapping to 9p24.1.

PRODUCT

Melan-A 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 Melan-A shRNA Plasmid (h): sc-35920-SH and Melan-A shRNA (h) Lentiviral Particles: sc-35920-V as alternate gene silencing products.

For independent verification of Melan-A (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-35920A, sc-35920B and sc-35920C.

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

Melan-A siRNA (h) is recommended for the inhibition of Melan-A 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

Melan-A (A103): sc-20032 is recommended as a control antibody for monitoring of Melan-A 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 Melan-A gene expression knockdown using RT-PCR Primer: Melan-A (h)-PR: sc-35920-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.