SANTA CRUZ BIOTECHNOLOGY, INC.

Pmel17 siRNA (m): sc-40645

BACKGROUND

Cytotoxic T lymphocytes (CTL's) recognize melanoma-associated antigens, which belong to three main groups. These groups include tumor-associated testis-specific antigens, melanocyte differentiation antigens and mutated or aberrantly expressed antigens, which are routinely used as markers to identify melanomas based on their binding to specific monoclonal antibodies. gp100, also designated ME20-M, ME20-S and PMEL 17, is classified as a melanocyte differentiation antigen and is expressed at low levels in normal cell lines and tissues, but is upregulated in melanocytes. gp100 is a highly glycosylated protein. It is also the product of proteolytic cleavage, which results in a secreted protein. gp100 is recognized by several monoclonal antibodies, including NKI-beteb, HMB-50 and HMB-45, which are used to diagnose melanomas. Therefore, gp100 is considered a potential target for immunotherapy of malignant melanoma.

REFERENCES

1. Kwon, B.S., et al. 1991. A melanocyte-specific gene, PMEL 17, maps near the silver coat color locus on mouse chromosome 10 and is in a syntenic region on human chromosome 12. Proc. Natl. Acad. Sci. USA 88: 9228-9232.
2. Maresh, G.A., et al. 1994. Differential processing and secretion of the melanoma-associated ME20 antigen. Arch. Biochem. Biophys. 311: 95-102.
3. Adema, G.J., et al. 1994. Molecular characterization of the melanocyte lineage-specific antigen gp100. J. Biol. Chem. 269: 20126-20133.
4. Adema, G.J., et al. 1996. PMEL 17 is recognised by monoclonal antibodies NKI-beteb, HMB-45 and HMB-50 and by anti-melanoma CTL. Br. J. Cancer 73: 1044-1048.
5. Schreurs, M.W., et al. 1997. Cloning, expression and tissue distribution of the murine homologue of the melanocyte lineage-specific antigen gp100. Melanoma Res. 7: 463-470.
6. Kirkin, A.F., et al. 1998. The immunogenic properties of melanoma-associated antigens recognized by cytotoxic T lymphocytes. Exp. Clin. Immunogenet. 15: 19-32.

CHROMOSOMAL LOCATION

Genetic locus: Pmel (mouse) mapping to 10 D3.

PRODUCT

Pmel17 siRNA (m) 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 Pmel17 shRNA Plasmid (m): sc-40645-SH and Pmel17 shRNA (m) Lentiviral Particles: sc-40645-V as alternate gene silencing products.

For independent verification of Pmel17 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-40645A, sc-40645B and sc-40645C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

Pmel17 siRNA (m) is recommended for the inhibition of Pmel17 expression in mouse 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

Pmel17 (C-2): sc-393094 is recommended as a control antibody for monitoring of Pmel17 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 Pmel17 gene expression knockdown using RT-PCR Primer: Pmel17 (m)-PR: sc-40645-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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