

PRAME siRNA (h): sc-37322

BACKGROUND

Several tumor-associated antigen families, such as MAGE, GAGE, PRAME and BAGE, are of particular interest in tumor immunology because their expression, with exception of testis and fetal tissues, seems to be restricted to tumor cells. The MAGE, BAGE and GAGE genes code for distinct antigens that are recognized by autologous cytolytic T lymphocytes. Many of these antigens represent suitable targets for tumor immunotherapy, since their expression in human melanoma cells is common and highly specific. PRAME (preferentially expressed antigen of melanoma) is a melanoma antigen recognized by cytotoxic T cells (CTLs) and is expressed in a variety of cancer cells, including leukemic cells. The PRAME gene is expressed at a high level in a very large fraction of tumors, such as melanomas, non small-cell lung carcinomas, sarcomas, head and neck tumors and renal carcinomas. Therefore, PRAME is a candidate for tumor immunotherapy, even though it is expressed at low levels in certain normal tissues.

REFERENCES

1. Li, J., et al. 1996. Expression of BAGE, GAGE, and MAGE genes in human gastric carcinoma. *Clin. Cancer Res.* 2: 1619-1625.
2. Dalerba, P., et al. 1998. High homogeneity of MAGE, BAGE, GAGE, tyrosinase and Melan-A/MART-1 gene expression in clusters of multiple simultaneous metastases of human melanoma: implications for protocol design of therapeutic antigen-specific vaccination strategies. *Int. J. Cancer* 77: 200-204.
3. van Baren, N., et al. 1998. PRAME, a gene encoding an antigen recognized on a human melanoma by cytolytic T cells, is expressed in acute leukaemia cells. *Br. J. Haematol.* 102: 1376-1379.
4. Pold, M., et al. 1999. Identification of a new, unorthodox member of the MAGE gene family. *Genomics* 59: 161-167.
5. Matsushita, M., et al. 2001. Quantitative monitoring of the PRAME gene for the detection of minimal residual disease in leukaemia. *Br. J. Haematol.* 112: 916-926.

CHROMOSOMAL LOCATION

Genetic locus: PRAME (human) mapping to 22q11.22.

PRODUCT

PRAME 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 PRAME shRNA Plasmid (h): sc-37322-SH and PRAME shRNA (h) Lentiviral Particles: sc-37322-V as alternate gene silencing products.

For independent verification of PRAME (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-37322A, sc-37322B and sc-37322C.

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

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

PRAME (D-12): sc-166480 is recommended as a control antibody for monitoring of PRAME 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 PRAME gene expression knockdown using RT-PCR Primer: PRAME (h)-PR: sc-37322-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.