

CML66 siRNA (m): sc-60418

BACKGROUND

The NUDCD1 gene encodes the 583 amino acid CML66 protein which demonstrates expression in a variety of solid tumor cell lines and leukemias. Expression of CML66 in normal tissues is restricted to testis and heart. The CML66 antibody is found in sera of 18-38% of patients with lung cancer, melanoma or prostate cancer. These findings indicate that CML66 may be immunogenic in a wide variety of malignancies and may be a target for antigen-specific immunotherapy. A short isoform of CML66, designated CML66-S, is a product of alternative splicing. CML66-S has the same C-terminus as full-length CML66 (also designated CML66-L), but has a unique N-terminus. CML66-S shows predominant expression in testis, and is also expressed in very low levels in tumor cells, while CML66 is expressed in tumor cells and testis.

REFERENCES

1. Yang, X.F., et al. 2001. CML66, a broadly immunogenic tumor antigen, elicits a humoral immune response associated with remission of chronic myelogenous leukemia. *Proc. Natl. Acad. Sci. USA* 98: 7492-7497.
2. Yan, Y., et al. 2004. A novel mechanism of alternative promoter and splicing regulates the epitope generation of tumor antigen CML66-L. *J. Immunol.* 172: 651-660.
3. You, Q., et al. 2004. Cloning, prokaryotic expression of CML66 and preparation of its polyclonal antibody. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 19: 582-584.
4. Wu, C.J., et al. 2005. Graft-versus-leukemia target antigens in chronic myelogenous leukemia are expressed on myeloid progenitor cells. *Clin. Cancer Res.* 11: 4504-4511.

CHROMOSOMAL LOCATION

Genetic locus: Nudcd1 (mouse) mapping to 15 B3.2.

PRODUCT

CML66 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CML66 shRNA Plasmid (m): sc-60418-SH and CML66 shRNA (m) Lentiviral Particles: sc-60418-V as alternate gene silencing products.

For independent verification of CML66 (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-60418A, sc-60418B and sc-60418C.

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

CML66 siRNA (m) is recommended for the inhibition of CML66 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 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

CML66 (C-4): sc-271650 is recommended as a control antibody for monitoring of CML66 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 CML66 gene expression knockdown using RT-PCR Primer: CML66 (m)-PR: sc-60418-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.