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CENP-M siRNA (m): sc-142266

BACKGROUND

During mitosis, the transient assembly of the kinetochore occurs on a platform known as the centromere, a specialized chromatin structure that is comprised of various centromere proteins (CENPs). There are two multi-protein centromere complexes, known as CENPA-NAC (nucleosome-associated) and CENPA-CAD (nucleosome distal), which interact with one another to facilitate both the assembly and the activity of the centromere. CENP-M (Centromere protein M), also known as CENPM, ICEN39 or PANE1, is a 180 amino acid centromeric protein that localizes to the nucleus in non-confluent cells and to the cytoplasm in dividing or confluent cells. One of several components of the CENPA-NAC complex, CENP-M plays a crucial role in the assembly of the kinetochore and the subsequent chromosome segregation and progression through mitosis. Additionally, CENP-M is thought to be involved in the incorporation of newly synthesized CENP-A into centromeres. Three isoforms of CENP-M exist due to alternative splicing events. Isoform 3 is expressed in B-lineage chronic lymphocytic leukemia (B-CLL) cells, suggesting a possible role in carcinogenesis.

REFERENCES

1. Online Mendelian Inheritance in Man, OMIM[™]. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 610152. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
2. Renou, J.P., et al. 2003. Identification of genes differentially expressed in mouse mammary epithelium transformed by an activated β -catenin. *Oncogene* 22: 4594-4610.
3. Brier, B., et al. 2004. The proliferation associated nuclear element (PANE1) is conserved between mammals and fish and preferentially expressed in activated lymphoid cells. *Gene Expr. Patterns* 4: 389-395.
4. Brickner, A.G., et al. 2006. The PANE1 gene encodes a novel human minor histocompatibility antigen that is selectively expressed in B-lymphoid cells and B-CLL. *Blood* 107: 3779-3786.
5. Izuta, H., et al. 2006. Comprehensive analysis of the ICEN (Interphase Centromere Complex) components enriched in the CENP-A chromatin of human cells. *Genes Cells* 11: 673-684.

CHROMOSOMAL LOCATION

Genetic locus: Cenpm (mouse) mapping to 15 E1.

PRODUCT

CENP-M 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 CENP-M shRNA Plasmid (m): sc-142266-SH and CENP-M shRNA (m) Lentiviral Particles: sc-142266-V as alternate gene silencing products.

For independent verification of CENP-M (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-142266A, sc-142266B and sc-142266C.

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

CENP-M siRNA (m) is recommended for the inhibition of CENP-M 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

CENP-M (B-7): sc-398754 is recommended as a control antibody for monitoring of CENP-M 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 CENP-M gene expression knockdown using RT-PCR Primer: CENP-M (m)-PR: sc-142266-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.