

nm23-H3 siRNA (h): sc-61205

BACKGROUND

The nm23 gene (metastasis inhibition factor nm23), a potential suppressor of metastasis, is expressed at a much lower level in highly metastatic cells than in cells with lower metastatic potential. Based on sequence analysis, nm23, also designated nucleoside diphosphate kinase A (NDK A) or tumor metastatic process-associated protein, appears to be highly related to nucleoside diphosphate kinases (NDP). NDP kinases A and B are identical to two isoforms of human nm23 homologs, nm23-H1 and nm23-H2, respectively. nm23-H2 is also identical in sequence to PuF, a transcription factor that binds to nucleoside-hypersensitive elements at positions 142 to 115 of the human c-Myc promoter. nm23-H3 and nm23-H4 are important for the synthesis of nucleoside triphosphates and may play a role in apoptosis induction and hematopoiesis. nm23-H3 is a 168 amino acid polypeptide. It is preferentially expressed during early stages of myeloid differentiation of highly purified CD34⁺ cells.

REFERENCES

1. Venturelli, D., et al. 1995. Overexpression of DR-nm23, a protein encoded by a member of the nm23 gene family, inhibits granulocyte differentiation and induces apoptosis in 32Dc13 myeloid cells. *Proc. Natl. Acad. Sci. USA* 92: 7435-7439.
2. Martinez, R., et al. 1997. Gene structure, promoter activity, and chromosomal location of the DR-nm23 gene, a related member of the nm23 gene family. *Cancer Res.* 57: 1180-1187.
3. Willems, R., et al. 1998. Decrease in nucleoside diphosphate kinase (NDPK/nm23) expression during hematopoietic maturation. *J. Biol. Chem.* 273: 13663-13668.
4. Daniels, R.J., et al. 2001. Sequence, structure and pathology of the fully annotated terminal 2 Mb of the short arm of human chromosome 16. *Hum. Mol. Genet.* 10: 339-352.
5. Masse, K., et al. 2002. Characterization of the nm23-M2, nm23-M3 and nm23-M4 mouse genes: comparison with their human orthologs. *Gene* 296: 87-97.
6. Willems, R., et al. 2002. Extracellular nucleoside diphosphate kinase NM23/NDPK modulates normal hematopoietic differentiation. *Exp. Hematol.* 30: 640-648.

CHROMOSOMAL LOCATION

Genetic locus: NME3 (human) mapping to 16p13.3.

PRODUCT

nm23-H3 siRNA (h) is a pool of 2 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 nm23-H3 shRNA Plasmid (h): sc-61205-SH and nm23-H3 shRNA (h) Lentiviral Particles: sc-61205-V as alternate gene silencing products.

For independent verification of nm23-H3 (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-61205A and sc-61205B.

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

nm23-H3 siRNA (h) is recommended for the inhibition of nm23-H3 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

nm23-H1/2/3 (E-11): sc-166937 is recommended as a control antibody for monitoring of nm23-H3 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 nm23-H3 gene expression knockdown using RT-PCR Primer: nm23-H3 (h)-PR: sc-61205-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.