

Nidogen siRNA (m): sc-43177

BACKGROUND

Basement membranes are the earliest extracellular matrices produced during embryogenesis. They are synthesized and incorporated into the supramolecular architecture of several components, including laminins, Collagen IV, Nidogen and proteoglycans. Nidogen/Entactin, a sulfated glycoprotein, acts as a link between the extracellular matrix molecules Laminin 1 and Collagen Type IV, and thereby participates in the assembly of basement membranes. Nidogen is a highly conserved member of the Nidogen family, which also includes Nidogen-2. Nidogen-2 has a high level of N- and O-glycosylation, and it interacts with Collagens Type I and IV and Perlecan at a comparable level to Nidogen. Nidogen is synthesized and secreted in primary and established mesenchymal peritubular cells and myoepithelial cells, and it affects adhesion of peritubular cells in an autocrine manner. Nidogen is expressed during embryonic and fetal development exclusively in fully developed basement membranes of the ectoderm and is not expressed in the developing endodermal basement membrane or in membranes disrupted during mesoderm formation. Nidogen also cooperates with Laminin 1 to regulate β -casein expression.

REFERENCES

- Schroen, D.J., et al. 1996. Interaction of mouse thymocytes and a thymocyte-like cell line with the ECM glycoprotein entactin. *Cell. Immunol.* 167: 141-149.
- Kofeldt, E., et al. 1998. Nidogen-2: a new basement membrane protein with diverse binding properties. *J. Mol. Biol.* 282: 99-109.
- Aumailley, M., et al. 2000. Altered synthesis of Laminin 1 and absence of basement membrane component deposition in Integrin β 1-deficient embryoid bodies. *J. Cell Sci.* 113: 259-268.
- Pujuguet, P., et al. 2000. Nidogen regulates Laminin 1-dependent mammary-specific gene expression. *J. Cell Sci.* 113: 849-858.
- Miosge, N., et al. 2000. Ultrastructural colocalization of Nidogen and Nidogen-2 with Laminin 1 in murine kidney basement membranes. *Histochem. Cell Biol.* 113: 115-124.
- Konrad, L., et al. 2000. Mesenchymal entactin-1 (Nidogen-1) is required for adhesion of peritubular cells of the rat testis *in vitro*. *Eur. J. Cell Biol.* 79: 112-120.

CHROMOSOMAL LOCATION

Genetic locus: Nid1 (mouse) mapping to 13 A1.

PRODUCT

Nidogen 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 Nidogen shRNA Plasmid (m): sc-43177-SH and Nidogen shRNA (m) Lentiviral Particles: sc-43177-V as alternate gene silencing products.

For independent verification of Nidogen (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-43177A, sc-43177B and sc-43177C.

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

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

Nidogen (C-7): sc-133175 is recommended as a control antibody for monitoring of Nidogen 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 Nidogen gene expression knockdown using RT-PCR Primer: Nidogen (m)-PR: sc-43177-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.