SANTA CRUZ BIOTECHNOLOGY, INC.

NGAL siRNA (h): sc-43969

BACKGROUND

In addition to the monomeric mammalian progelatinase, two additional forms of progelatinase have been identified. The shorter of these additional forms is a covalently linked, disulfide-bridged protein that heterodimerizes with a short protein; an α2-Microglobulin-related protein known as neutrophil gelatinase-associated lipocalin (NGAL), which is moderately expressed in breast and lung tissues. NGAL belongs to the lipocalin family and has a high degree of similarity with rat α2-Microglobulin-related protein and mouse protein 24p3. NGAL is able to bind a derivative of the bacterial chemotactic peptide, suggesting that it has important immuno-modulatory functions. NGAL has been described as an inflammatory protein; it is released into the circulation as a result of the inflammatory activation of leukocytes initiated by the extra-corporeal circulation. In addition, NGAL synthesis is induced in epithelial cells in inflammatory and neoplastic colorectal diseases. In conclusion, NGAL may serve as a scavenger of bacterial products to function in the anti-inflammatory process.

REFERENCES

1. Triebel, S., et al. 1992. A 25 kDa α2-Microglobulin-related protein is a component of the 125 kDa form of human gelatinase. FEBS Lett. 314: 386-388.
2. Kjeldsen, L., et al. 1993. Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. J. Biol. Chem. 268: 10425-10432.
3. Bundgaard, J.R., et al. 1994. Molecular cloning and expression of a cDNA encoding NGAL: a lipocalin expressed in human neutrophils. Biochem. Biophys. Res. Commun. 202: 1468-1475.
4. Nielsen, B.S., et al. 1996. Induction of NGAL synthesis in epithelial cells of human colorectal neoplasia and inflammatory bowel diseases. Gut 38: 414-420.
5. Stoesz, S.P., et al. 1998. Heterogeneous expression of the lipocalin NGAL in primary breast cancers. Int. J. Cancer 79: 565-572.
6. Jönsson, P., et al. 1999. Extracorporeal circulation causes release of neutrophil gelatinase-associated lipocalin (NGAL). Mediators Inflamm. 8: 169-171.

CHROMOSOMAL LOCATION

Genetic locus: LCN2 (human) mapping to 9q34.11.

PRODUCT

NGAL 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NGAL shRNA Plasmid (h): sc-43969-SH and NGAL shRNA (h) Lentiviral Particles: sc-43969-V as alternate gene silencing products.

For independent verification of NGAL (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-43969A, sc-43969B and sc-43969C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

NGAL siRNA (h) is recommended for the inhibition of NGAL 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 µM in 66 µl. 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

NGAL (H-4): sc-518095 is recommended as a control antibody for monitoring of NGAL 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 NGAL gene expression knockdown using RT-PCR Primer: NGAL (h)-PR: sc-43969-PR (20 µl, 540 bp). 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.