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LEKTI siRNA (h): sc-45358

BACKGROUND

Lympho-epithelial Kazal-type inhibitor (LEKTI) is a serine protease inhibitor which protects mucous epithelia against microbial attack and inflammation. LEKTI is a marker of epithelial differentiation and expresses strongly in the granular and uppermost spinous layers of the epidermis and differentiated layers of stratified epithelia. Defects in SPINK5, the gene encoding LEKTI, are the cause of Netherton syndrome, a severe autosomal recessive disorder characterized by atopic dermatitis, hayfever and other conditions.

REFERENCES

- Magert, H.J., et al. 1999. LEKTI, a novel 15-domain type of human serine proteinase inhibitor. *J. Biol. Chem.* 274: 21499-21502.
- Walden, M., et al. 2002. Biochemical features, molecular biology and clinical relevance of the human 15-domain serine proteinase inhibitor LEKTI. *Biol. Chem.* 383: 1139-1141.
- Magert, H.J., et al. 2002. LEKTI: a multidomain serine proteinase inhibitor with pathophysiological relevance. *Int. J. Biochem. Cell. Biol.* 34: 573-576.
- Lauber, T., et al. 2003. Homologous proteins with different folds: the three-dimensional structures of domains 1 and 6 of the multiple Kazal-type inhibitor LEKTI. *J. Mol. Biol.* 328: 205-219.
- Mitsudo, K., et al. 2003. Inhibition of serine proteinases plasmin, trypsin, subtilisin A, cathepsin G and elastase by LEKTI: a kinetic analysis. *Biochemistry* 42: 3874-3881.
- Bitoun, E., et al. 2003. LEKTI proteolytic processing in human primary keratinocytes, tissue distribution and defective expression in Netherton syndrome. *Hum. Mol. Genet.* 12: 2417-2430.

CHROMOSOMAL LOCATION

Genetic locus: SPINK5 (human) mapping to 5q32.

PRODUCT

LEKTI 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see LEKTI shRNA Plasmid (h): sc-45358-SH and LEKTI shRNA (h) Lentiviral Particles: sc-45358-V as alternate gene silencing products.

For independent verification of LEKTI (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-45358A, sc-45358B and sc-45358C.

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

LEKTI siRNA (h) is recommended for the inhibition of LEKTI 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

LEKTI (E-9): sc-137109 is recommended as a control antibody for monitoring of LEKTI 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 LEKTI gene expression knockdown using RT-PCR Primer: LEKTI (h)-PR: sc-45358-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.