SANTA CRUZ BIOTECHNOLOGY, INC.

Cytokeratin 3 siRNA (h): sc-60500

BACKGROUND

Cytokeratins comprise a diverse group of intermediate filament proteins that are expressed as pairs in both keratinized and non-keratinized epithelial tissue. The cytokeratin proteins play a critical role in differentiation, as well as tissue specialization and function, to maintain the overall structural integrity of epithelial cells. Cytokeratins are also useful markers in identifying the origin of metastatic tumors. There are two types of cytokeratins: types I and II. Type I cytokeratins are acidic proteins, whereas type II cytokeratins are neutral or basic proteins. Cytokeratin 3 is a type II cytokeratin expressed in the corneal epithelium along with Cytokeratin 12. Dominant-negative mutations in the Cytokeratin 3 and Cytokeratin 12 genes cause Meesmann corneal dystrophy (MCD), an autosomal dominant disease that is characterized by weakness of the anterior corneal epithelium.

REFERENCES

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2. Schermer, A., et al. 1986. Differentiation-related expression of a major 64 kDa corneal keratin *in vivo* and in culture suggests limbal location of corneal epithelial stem cells. J. Cell Biol. 103: 49-62.
3. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 148043. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
4. Kawakita, T., et al. 2005. Intrastromal invasion by limbal epithelial cells is mediated by epithelial-mesenchymal transition activated by air exposure. Am. J. Pathol. 167: 381-393.
5. Ma, Y., et al. 2006. Reconstruction of chemically burned rat corneal surface by bone marrow-derived human mesenchymal stem cells. Stem Cells 24: 315-321.
6. Miyashita, H., et al. 2006. Collagen-immobilized poly (vinyl alcohol) as an artificial cornea scaffold that supports a stratified corneal epithelium. J. Biomed. Mater. Res. B, Appl. Biomater. 76: 56-63.
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CHROMOSOMAL LOCATION

Genetic locus: KRT3 (human) mapping to 12q13.13.

PRODUCT

Cytokeratin 3 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 Cytokeratin 3 shRNA Plasmid (h): sc-60500-SH and Cytokeratin 3 shRNA (h) Lentiviral Particles: sc-60500-V as alternate gene silencing products.

For independent verification of Cytokeratin 3 (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-60500A, sc-60500B and sc-60500C.

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

Cytokeratin 3 siRNA (h) is recommended for the inhibition of Cytokeratin 3 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

Cytokeratin 3/2p (AE5): sc-80000 is recommended as a control antibody for monitoring of Cytokeratin 3 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 Cytokeratin 3 gene expression knockdown using RT-PCR Primer: Cytokeratin 3 (h)-PR: sc-60500-PR (20 µl). 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.