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Filaggrin siRNA (h): sc-43364

BACKGROUND

Profilaggrin is a large, insoluble, highly phosphorylated precursor protein containing several tandem copies of a 324 amino acid stretch. Mammalian profilaggrin is a major protein component of keratohyalin granules in the living cells of the epidermis. Keratohyalin granules contribute to the keratin content of dead cornified cells. During terminal differentiation of the epidermis, profilaggrin is proteolytically processed into active Filaggrin molecules that promote aggregation and disulfide-bond formation of keratin intermediate filaments. Active Filaggrin is present at a level of the epidermis where keratinocytes are in transition between the live nucleated granular layer and the anucleate cornified layer, suggesting that Filaggrin aids in the terminal differentiation process by facilitating apoptotic machinery.

REFERENCES

- McKinley-Grant, L.J., et al. 1989. Characterization of a cDNA clone encoding human Filaggrin and localization of the gene to chromosome region 1q21. *Proc. Natl. Acad. Sci. USA* 86: 4848-4852.
- Gan, S.Q., et al. 1990. Organization, structure and polymorphisms of the human profilaggrin gene. *Biochemistry* 29: 9432-9440.
- Takahashi, M., et al. 1996. Filaggrin linker segment peptide and cystatin α are parts of a complex of the cornified envelope of epidermis. *Arch. Biochem. Biophys.* 329: 123-126.
- Gerritsen, M.J., et al. 1997. Recruitment of cycling epidermal cells and expression of Filaggrin, involucrin and Tenascin in the margin of the active psoriatic plaque, in the uninvolved skin of psoriatic patients and in the normal healthy skin. *J. Dermatol. Sci.* 14: 179-188.
- Online Mendelian Inheritance in Man, OMIM[™]. 1998. Johns Hopkins University, Baltimore, MD. MIM Number: 135940. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
- Kuechle, M.K., et al. 2000. Inducible expression of Filaggrin increases keratinocyte susceptibility to apoptotic cell death. *Cell Death Differ.* 7: 566-573.

CHROMOSOMAL LOCATION

Genetic locus: FLG (human) mapping to 1q21.3.

PRODUCT

Filaggrin 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 Filaggrin shRNA Plasmid (h): sc-43364-SH and Filaggrin shRNA (h) Lentiviral Particles: sc-43364-V as alternate gene silencing products.

For independent verification of Filaggrin (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-43364A, sc-43364B and sc-43364C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

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

Filaggrin (AKH1): sc-66192 is recommended as a control antibody for monitoring of Filaggrin 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 Filaggrin gene expression knockdown using RT-PCR Primer: Filaggrin (h)-PR: sc-43364-PR (20 μ l, 600 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Kovacs, D., et al. 2020. Bovine colostrum induces the differentiation of human primary keratinocytes. *FASEB J.* E-published.

RESEARCH USE

For research use only, not for use in diagnostic procedures.