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Filaggrin shRNA (h) Lentiviral Particles: sc-43364-V

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.

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CHROMOSOMAL LOCATION

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

PRODUCT

Filaggrin shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Filaggrin siRNA (h): sc-43364 and Filaggrin shRNA Plasmid (h): sc-43364-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80°C . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

Filaggrin shRNA (h) Lentiviral Particles is recommended for the inhibition of Filaggrin expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

Filaggrin (H-300): sc-30229 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker[™] compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker[™] Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz[™] Mounting Medium: sc-24941.

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 $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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