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Cytokeratin 12 siRNA (h): sc-43306

BACKGROUND

Cytokeratins comprise a diverse group of intermediate filament proteins (IFPs) that are expressed as pairs in both keratinized and non-keratinized epithelial tissue, where they constitute up to 85% of mature keratinocytes in the vertebrate epidermis. Cytokeratins play a critical role in differentiation and tissue specialization and function to maintain the overall structural integrity of epithelial cells. The α -helical, coiled-coil dimers associate laterally end-to-end to form 10 nm diameter filaments. Cytokeratins are useful markers of tissue differentiation, and Cytokeratin 12 is a distinct marker of tissue differentiation in the developing cornea. Cytokeratin 12 and Cytokeratin 3 are expressed in the corneal epithelium, where Cytokeratin 12 provides structural integrity to an otherwise fragile cornea. Human Cytokeratin 12 gene mutations cause Meesmann's corneal dystrophy, an autosomal dominant disorder characterized by corneal epithelia fragility and intra-epithelial microcysts.

REFERENCES

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CHROMOSOMAL LOCATION

Genetic locus: KRT12 (human) mapping to 17q21.2.

PRODUCT

Cytokeratin 12 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 Cytokeratin 12 shRNA Plasmid (h): sc-43306-SH and Cytokeratin 12 shRNA (h) Lentiviral Particles: sc-43306-V as alternate gene silencing products.

For independent verification of Cytokeratin 12 (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-43306A, sc-43306B and sc-43306C.

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

Cytokeratin 12 siRNA (h) is recommended for the inhibition of Cytokeratin 12 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

Cytokeratin 12 (E-8): sc-515882 is recommended as a control antibody for monitoring of Cytokeratin 12 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 Cytokeratin 12 gene expression knockdown using RT-PCR Primer: Cytokeratin 12 (h)-PR: sc-43306-PR (20 μ l, 428 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.