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Cytokeratin 12 shRNA (h) Lentiviral Particles: sc-43306-V

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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5. Quillien, V., et al. 1995. Serum and tissue distribution of a fragment of Cytokeratin 19 (CYFRA 21-1) in lung cancer patients. *Anticancer Res.* 15: 2857-2863.
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CHROMOSOMAL LOCATION

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

PRODUCT

Cytokeratin 12 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 Cytokeratin 12 siRNA (h): sc-43306 and Cytokeratin 12 shRNA Plasmid (h): sc-43306-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

Cytokeratin 12 shRNA (h) Lentiviral Particles is recommended for the inhibition of Cytokeratin 12 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

Cytokeratin 12 (N-16): sc-17098 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 $55-60^\circ\text{C}$ 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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