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Cytokeratin 18 shRNA (m) Lentiviral Particles: sc-45406-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. Cytokeratins play a critical role in differentiation and tissue specialization and function to maintain the overall structural integrity of epithelial cells. Cytokeratins have been found to be useful markers of tissue differentiation, which is directly applicable to the characterization of malignant tumors. For example, Cytokeratins 10 and 13 are highly expressed in a subset of squamous cell carcinomas while Cytokeratin 18 is expressed in a majority of adenocarcinomas and basal cell carcinomas. Cytokeratin 18 contains two major phosphorylation sites, Ser 33 and Ser 52. Phosphorylation of Ser 18 is essential for the association of Cytokeratin 18 with 14-3-3 proteins and is involved in keratin organization and distribution.

REFERENCES

1. Lauerová, L., et al. 1988. Novel monoclonal antibodies defining epitope of human Cytokeratin 18 molecule. *Hybridoma* 7: 495-504.
2. Vojtesek, B., et al. 1989. Monoclonal antibodies recognizing different epitopes of Cytokeratin 18. *Folia Biol.* 35: 373-382.
3. van der Velden, L.A., et al. 1993. Cytokeratin expression in normal and (pre)malignant head and neck epithelia: an overview. *Head Neck* 15: 133-146.
4. Silen, A., et al. 1994. Evaluation of a new tumor marker for Cytokeratin 8 and 18 fragments in healthy individuals and prostate cancer patients. *Prostate* 24: 326-332.
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.

CHROMOSOMAL LOCATION

Genetic locus: Krt18 (mouse) mapping to 15 F3.

PRODUCT

Cytokeratin 18 shRNA (m) 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 18 siRNA (m): sc-45406 and Cytokeratin 18 shRNA Plasmid (m): sc-45406-SH as alternate gene silencing products.

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.

APPLICATIONS

Cytokeratin 18 shRNA (m) Lentiviral Particles is recommended for the inhibition of Cytokeratin 18 expression in mouse 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 18 (H-80): sc-28264 is recommended as a control antibody for monitoring of Cytokeratin 18 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 Cytokeratin 18 gene expression knockdown using RT-PCR Primer: Cytokeratin 18 (m)-PR: sc-45406-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

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.

PROTOCOLS

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