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Cytokeratin 18 siRNA (m2): sc-43287

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 expressed highly 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 on 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. Lauerova, 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 No.18. *Folia Biol.* 35: 373-382.
3. Vander 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 siRNA (m2) 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 18 shRNA Plasmid (m2): sc-43287-SH and Cytokeratin 18 shRNA (m2) Lentiviral Particles: sc-43287-V as alternate gene silencing products.

For independent verification of Cytokeratin 18 (m2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-43287A, sc-43287B and sc-43287C.

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.

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 18 siRNA (m2) is recommended for the inhibition of Cytokeratin 18 expression in mouse 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 18 (RGE53): sc-32329 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 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 18 gene expression knockdown using RT-PCR Primer: Cytokeratin 18 (m2)-PR: sc-43287-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.