



Cytokeratin 6A siRNA (h): sc-105266

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, which exist as α -helical coiled-coil dimers that associate laterally end-to-end to form 10 nm diameter filaments, play a critical role in differentiation and tissue specialization and function to maintain the overall structural integrity of epithelial cells. Cytokeratins, which are useful markers of tissue differentiation, also aid in the characterization of malignant tumors. IL-1 and TNF α induce transcription of Cytokeratin 6 in epidermal keratinocytes via the C/EBP β transcription factor. In humans, multiple isoforms of Cytokeratin 6 (6A-6F), encoded by several highly homologous genes, have distinct tissue expression patterns. Cytokeratin 6A is the dominant form in epithelial tissue. The gene encoding human Cytokeratin 6A maps to chromosome 12q13.13 and mutations in this gene are linked to several inheritable hair and skin pathologies.

REFERENCES

1. 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.
2. Marceau, N. and Loranger, A. 1995. Cytokeratin expression, fibrillar organization and subtle function in liver cells. *Biochem. Cell Biol.* 73: 619-625.
3. Fuchs, E. 1995. Keratins and the skin. *Annu. Rev. Cell Dev. Biol.* 11: 123-153.
4. 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.
5. Mukhopadhyay, T. and Roth, J.A. 1996. Functional inactivation of p53 by antisense RNA induces invasive ability of lung carcinoma cells and down-regulates cytokeratin synthesis. *Anticancer Res.* 16: 1683-1689.
6. Machesney, M., et al. 1998. Activated keratinocytes in the epidermis of hypertrophic scars. *Am. J. Pathol.* 152: 1133-1141.
7. Porter, R.M., et al. 2000. K15 expression implies lateral differentiation within stratified epithelial basal cells. *Lab. Invest.* 80: 1701-1710.

CHROMOSOMAL LOCATION

Genetic locus: KRT6A (human) mapping to 12q13.13.

PRODUCT

Cytokeratin 6A 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 6A shRNA Plasmid (h): sc-105266-SH and Cytokeratin 6A shRNA (h) Lentiviral Particles: sc-105266-V as alternate gene silencing products.

For independent verification of Cytokeratin 6A (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-105266A, sc-105266B and sc-105266C.

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 6A siRNA (h) is recommended for the inhibition of Cytokeratin 6A 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 6 (B-7): sc-514520 is recommended as a control antibody for monitoring of Cytokeratin 6A 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 6A gene expression knockdown using RT-PCR Primer: Cytokeratin 6A (h)-PR: sc-105266-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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