

Keratin 74 siRNA (h): sc-95780

BACKGROUND

The Keratin multigene family is made of "soft" epithelial cytokeratins and "hard" hair Keratins. While the epithelial cytokeratins are involved in the layering and formation of epithelia, the hair Keratins are responsible for creating nails and hair. There are two types of Keratins: the acidic class I Keratin proteins and the basic/neutral class II Keratin proteins. Keratin 74 (KRT74), also known as K6IRS4, KRT5C or KRT6IRS4, is a 529 amino acid protein that is highly expressed in scalp hair follicles. Specifically, Keratin 74 is found in the Huxley layer of the inner root sheath (IRS). Woolly hair autosomal dominant (ADWH) is a rare disorder caused by defects in the Keratin 74 gene which causes fine and tightly curled hair that stops growing after a few inches. Only affecting the scalp, progressive hair loss begins at early adulthood and complete baldness occurs after 30 years. The gene encoding Keratin 74 maps to human chromosome 12.

REFERENCES

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2. Rogers, M.A., Winter, H., Langbein, L., Wolf, C. and Schweizer, J. 2000. Characterization of a 300 kbp region of human DNA containing the type II hair keratin gene domain. *J. Invest. Dermatol.* 114: 464-472.
3. Langbein, L., Rogers, M.A., Winter, H., Praetzel, S. and Schweizer, J. 2001. The catalog of human hair keratins. II. Expression of the six type II members in the hair follicle and the combined catalog of human type I and II keratins. *J. Biol. Chem.* 276: 35123-35132.
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5. Langbein, L. and Schweizer, J. 2005. Keratins of the human hair follicle. *Int. Rev. Cytol.* 243: 1-78.
6. Rogers, M.A., Edler, L., Winter, H., Langbein, L., Beckmann, I. and Schweizer, J. 2005. Characterization of new members of the human type II keratin gene family and a general evaluation of the keratin gene domain on chromosome 12q13.13. *J. Invest. Dermatol.* 124: 536-544.

CHROMOSOMAL LOCATION

Genetic locus: KRT74 (human) mapping to 12q13.13.

PRODUCT

Keratin 74 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 Keratin 74 shRNA Plasmid (h): sc-95780-SH and Keratin 74 shRNA (h) Lentiviral Particles: sc-95780-V as alternate gene silencing products.

For independent verification of Keratin 74 (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-95780A, sc-95780B and sc-95780C.

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

Keratin 74 siRNA (h) is recommended for the inhibition of Keratin 74 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

Keratin 74 (E-6): sc-390340 is recommended as a control antibody for monitoring of Keratin 74 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 Keratin 74 gene expression knockdown using RT-PCR Primer: Keratin 74 (h)-PR: sc-95780-PR (20 μ l). 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.