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involucrin siRNA (m): sc-43367

BACKGROUND

Involucrin is a precursor protein of the keratinocyte cornified envelope, which is formed beneath the inner surface of the cell membrane during terminal differentiation. Involucrin first appears in the cell cytosol but ultimately becomes cross-linked to membrane proteins by transglutaminase. During keratinocyte terminal differentiation, glutamine residues of involucrin become covalently cross-linked to other envelope precursors via covalent epsilon-(γ -glutamyl) lysine bonds. Moreover, its large size allows involucrin to cross-link molecules that are separated by substantial distances in the cornified envelope. These properties allow a single involucrin molecule to form multiple cross-links, in multiple spatial planes, with other envelope precursors. Involucrin is specifically expressed in Chinese hamster ovarian cells (fibroblasts), PtK2 rat kangaroo kidney cells (simple epithelial), and rat epidermal keratinocytes (stratifying squamous epithelial).

REFERENCES

- Eckert, R.L., et al. 1986. Structure and evolution of the human involucrin gene. *Cell* 46: 583-589.
- Rorke, E.A. et al. 1991. Stable expression of transfected human involucrin gene in various cell types: evidence for *in situ* cross-linking by type I and type II transglutaminase. *J. Invest. Dermatol.* 97: 543-548.
- Yaffe, M.B., et al. 1992. Biophysical characterization of involucrin reveals a molecule ideally suited to function as an intermolecular cross-bridge of the keratinocyte cornified envelope. *J. Biol. Chem.* 267: 12233-12238.
- Crish, J.F., et al. 1993. Tissue-specific and differentiation-appropriate expression of the human involucrin gene in transgenic mice: an abnormal epidermal phenotype. *Differentiation* 53: 191-200.
- Takahashi, H., et al. 1995. Repression of involucrin gene expression by transcriptional enhancer factor 1 (TEF-1). *Arch. Dermatol. Res.* 287: 740-746.

CHROMOSOMAL LOCATION

Genetic locus: Ivl (mouse) mapping to 3 F1.

PRODUCT

involucrin siRNA (m) 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 involucrin shRNA Plasmid (m): sc-43367-SH and involucrin shRNA (m) Lentiviral Particles: sc-43367-V as alternate gene silencing products.

For independent verification of involucrin (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-43367A, sc-43367B and sc-43367C.

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

involucrin siRNA (m) is recommended for the inhibition of involucrin 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

involucrin (SY5): sc-21748 is recommended as a control antibody for monitoring of involucrin 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor involucrin gene expression knockdown using RT-PCR Primer: involucrin (m)-PR: sc-43367-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.