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# involucrin shRNA (m) Lentiviral Particles: sc-43367-V

## 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

1. Eckert, R.L., et al. 1986. Structure and evolution of the human involucrin gene. *Cell* 46: 583-589.
2. 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.
3. 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.
4. 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.
5. 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 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 µl frozen stock containing  $1.0 \times 10^6$  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 involucrin siRNA (m): sc-43367 and involucrin shRNA Plasmid (m): sc-43367-SH as alternate gene silencing products.

## 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.

## APPLICATIONS

involucrin shRNA (m) Lentiviral Particles is recommended for the inhibition of involucrin expression in mouse cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing  $1.0 \times 10^6$  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

involucrin (M-116): sc-28558 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).

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 involucrin gene expression knockdown using RT-PCR Primer: involucrin (m)-PR: sc-43367-PR (20 µl). 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.

## 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.

## PROTOCOLS

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