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desmoplakin I/II shRNA (m) Lentiviral Particles: sc-45911-V

BACKGROUND

Desmosomes are major cell adhesion junctions that are particularly prominent in the epidermis and in cardiac tissue and are important for the rigidity and strength of the cell. The desmosome consists of several proteins, of which desmoplakin is the most abundant. Desmoplakin plays an important role in the attachment of the filaments to the desmosome. Specifically, desmoplakin interacts with plakophilin 1 (PKP1), PKP2 or PKP3, or combinations thereof, to selectively recruit plakophilins to desmosomal plaques. Desmoplakin has also been shown to function as a transglutaminase substrate *in vitro*, suggesting that it may participate in cell adhesion at the intraepidermal level. Desmoplakin exists as a two-stranded coil structure. Alternative splicing gives rise to two isoforms, desmoplakin I and II, which differ by 600 amino acids.

REFERENCES

- Green, K.J., et al. 1990. Structure of the human desmoplakins: implications for function in the desmosomal plaque. *J. Biol. Chem.* 265: 2603-2612. Erratum: *J. Biol. Chem.* 265: 11406-11407.
- Norgett, E.E., et al. 2000. Recessive mutation in desmoplakin disrupts desmoplakin-intermediate filament interactions and causes dilated cardiomyopathy, woolly hair and keratoderma. *Hum. Mol. Genet.* 9: 2761-2766.
- Hofmann, I., et al. 2000. Interaction of plakophilins with desmoplakin and intermediate filament proteins: an *in vitro* analysis. *J. Cell. Sci.* 113: 2471-2483.
- Esposito, C., et al. 2000. Implication of tissue transglutaminase and desmoplakin in cell adhesion mechanism in human epidermis. *Mol. Cell. Biochem.* 206: 57-65.

CHROMOSOMAL LOCATION

Genetic locus: Dsp (mouse) mapping to 13 A3.3.

PRODUCT

desmoplakin I/II 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 μ l frozen stock containing 1.0×10^6 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 desmoplakin I/II siRNA (m): sc-45911 and desmoplakin I/II shRNA Plasmid (m): sc-45911-SH as alternate gene silencing products.

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.

APPLICATIONS

desmoplakin I/II shRNA (m) Lentiviral Particles is recommended for the inhibition of desmoplakin I/II expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

desmoplakin I/II (H-300): sc-33555 is recommended as a control antibody for monitoring of desmoplakin I/II 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 desmoplakin I/II gene expression knockdown using RT-PCR Primer: desmoplakin I/II (m)-PR: sc-45911-PR (20 μ l). 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.

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.

PROTOCOLS

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