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envoplakin shRNA (h) Lentiviral Particles: sc-43412-V

BACKGROUND

Paraneoplastic pemphigus (PNP) is an autoimmune blistering disease that is associated with underlying neoplasms. PNP sera react with multiple plakins family proteins, among which only envoplakin and periplakin are constantly detected. Envoplakin, a membrane-associated precursor of the epidermal cornified envelope and desmosomes, is a member of the plakins family of proteins. Envoplakin is expressed in epidermal and esophageal keratinocytes and nonepithelial stratified squamous epithelia, but not in simple epithelia or nonepithelial cells. Envoplakin colocalizes with desmoplakin at desmosomes and on keratin filaments throughout the differentiated layers of the epidermis, but mainly accumulates in nuclear and cytoplasmic aggregates with associated intermediate filaments. The envoplakin rod domain is required for aggregation and the linker domain is required for intermediate filament association. The distribution of envoplakin at the interdesmosomal plasma membrane depends on heterodimerization with periplakin.

REFERENCES

1. Ruhrberg, C., et al. 1996. Chromosomal localization of the human envoplakin gene (EVPL) to the region of the tylosis oesophageal cancer gene (TOCG) on 17q25. *Genomics* 37: 381-385.
2. Ruhrberg, C., et al. 1996. Envoplakin, a novel precursor of the cornified envelope that has homology to desmoplakin. *J. Cell Biol.* 134: 715-29.
3. Chorzelski, T., et al. 1999. Paraneoplastic pemphigus associated with Castleman tumor, myasthenia gravis and bronchiolitis obliterans. *J. Am. Acad. Dermatol.* 41: 393-400.
4. Risk, J.M., et al. 1999. Envoplakin, a possible candidate gene for focal NEPPK/esophageal cancer (TOC): the integration of genetic and physical maps of the TOC region on 17q25. *Genomics* 59: 234-242.

CHROMOSOMAL LOCATION

Genetic locus: EVPL (human) mapping to 17q25.1.

PRODUCT

envoplakin shRNA (h) 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 1.0×10^6 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 envoplakin siRNA (h): sc-43412 and envoplakin shRNA Plasmid (h): sc-43412-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.

PROTOCOLS

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

APPLICATIONS

envoplakin shRNA (h) Lentiviral Particles is recommended for the inhibition of envoplakin expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl 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

envoplakin (F-4): sc-137033 is recommended as a control antibody for monitoring of envoplakin 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-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor envoplakin gene expression knockdown using RT-PCR Primer: envoplakin (h)-PR: sc-43412-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.