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envoplakin siRNA (h): sc-43412

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.
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4. Chorzelski, T., et al. 1999. Paraneoplastic pemphigus associated with Castleman tumor, myasthenia gravis and bronchiolitis obliterans. *J. Am. Acad. Dermatol.* 41: 393-400.
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6. Maatta, A., et al. 2000. Structure and regulation of the envoplakin gene. *J. Biol. Chem.* 275: 19857-19865.
7. Di Colandrea, T., et al. 2000. Subcellular distribution of envoplakin and periplakin: insights into their role as precursors of the epidermal cornified envelope. *J. Cell Biol.* 15: 573-586.

CHROMOSOMAL LOCATION

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

PRODUCT

envoplakin 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 envoplakin shRNA Plasmid (h): sc-43412-SH and envoplakin shRNA (h) Lentiviral Particles: sc-43412-V as alternate gene silencing products.

For independent verification of envoplakin (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-43412A, sc-43412B and sc-43412C.

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

envoplakin siRNA (h) is recommended for the inhibition of envoplakin 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

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 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 envoplakin gene expression knockdown using RT-PCR Primer: envoplakin (h)-PR: sc-43412-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.