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Caldesmon shRNA (h2) Lentiviral Particles: sc-44291-V

BACKGROUND

Caldesmon, Filamin 1, Nebulin and Villin are differentially expressed and regulated Actin binding proteins. Both muscular and non-muscular forms of Caldesmon have been identified and each has been shown to bind to Actin as well as to calmodulin and Myosin. Alternative splicing of the gene encoding Caldesmon results in five isoforms. Muscular Caldesmon (isoform 1), also designated high molecular weight Caldesmon or H-Caldesmon (H-CAD), is expressed predominantly on thin filaments in smooth muscle. Non-muscular Caldesmon (isoforms 2-5), also designated low molecular weight Caldesmon or L-Caldesmon (L-CAD), is widely expressed in non-muscle tissues and cells. Filamin 1, which is ubiquitously expressed and exists as a homodimer, functions to crosslink Actin to filaments. Nebulin is a large filamentous protein specific to muscle tissue that may function as a ruler for filament length. Several isoforms of Nebulin are produced by alternative exon usage. Villin is Ca²⁺-regulated and is the major structural component of the brush border of absorptive cells.

REFERENCES

1. Weihing, R.R. 1988. Actin-binding and dimerization domains of HeLa cell Filamin. *Biochemistry* 27: 1865-1869.
2. Marston, S., et al. 1992. Caldesmon binds to smooth muscle myosin and myosin rod and crosslinks thick filaments to actin filaments. *J. Muscle Res. Cell Motil.* 13: 206-218.
3. Maunoury, R., et al. 1992. Developmental regulation of Villin gene expression in the epithelial cell lineages of mouse digestive and urogenital tracts. *Development* 115: 717-728.
4. Labeit, S., et al. 1995. The complete primary structure of human Nebulin and its correlation to muscle structure. *J. Mol. Biol.* 248: 308-315.

CHROMOSOMAL LOCATION

Genetic locus: CALD1 (human) mapping to 7q33.

PRODUCT

Caldesmon shRNA (h2) 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 Caldesmon siRNA (h2): sc-44291 and Caldesmon shRNA Plasmid (h2): sc-44291-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

Caldesmon shRNA (h2) Lentiviral Particles is recommended for the inhibition of Caldesmon expression in human 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

Caldesmon (A-2): sc-271222 is recommended as a control antibody for monitoring of Caldesmon 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 IgM-HRP: sc-2064 (dilution range: 1:500-1:5,000), TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgM-FITC: sc-2082 (dilution range: 1:100-1:400) or goat anti-mouse IgM-TR: sc-2983 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Caldesmon gene expression knockdown using RT-PCR Primer: Caldesmon (h2)-PR: sc-44291-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.