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transgelin shRNA (h) Lentiviral Particles: sc-44163-V

BACKGROUND

Transgelin, also designated SM22 α , is expressed abundantly in smooth muscle cells. The human transgelin gene (designated TAGLN), which is located on chromosome 11q23.3, encodes a 201 amino acid protein that contains nuclear factor-binding motifs known to regulate transcription in smooth muscle. During embryogenesis, transgelin is expressed in smooth, cardiac and skeletal muscle, but is restricted during late fetal development and adulthood to all vascular and visceral smooth muscle cells and low levels of expression in heart. Transgelin is downregulated in several transformed cell lines, indicating that a reduction of transgelin expression may be an early indicator of the onset of transformation. Transgelin also binds Actin, causing Actin fibers to gel within minutes of binding. Binding of transgelin to Actin occurs at a ratio of 1:6 Actin monomers.

REFERENCES

1. Shapland, C., et al. 1993. Purification and properties of transgelin: a transformation and shape change sensitive Actin-gelling protein. *J. Cell Biol.* 121: 1065-1073.
2. Kobayashi, R., et al. 1994. Purification, characterization and partial sequence analysis of a new 25 kDa Actin-binding protein from bovine aorta: an SM22 homolog. *Biochem. Biophys. Res. Commun.* 198: 1275-1280.
3. Li, L., et al. 1997. Evidence for serum response factor-mediated regulatory networks governing SM22a transcription in smooth, skeletal and cardiac muscle cells. *Dev. Biol.* 187: 311-321.
4. Lawson, D., et al. 1997. Fibroblast transgelin and smooth muscle SM22a are the same protein, the expression of which is downregulated in many cell lines. *Cell Motil. Cytoskeleton* 38: 250-257.
5. Camoretti-Mercado, B., et al. 1998. Expression and cytogenetic localization of the human SM22 gene (TAGLN). *Genomics* 49: 452-457.

CHROMOSOMAL LOCATION

Genetic locus: TAGLN (human) mapping to 11q23.3.

PRODUCT

transgelin 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 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 transgelin siRNA (h): sc-44163 and transgelin shRNA Plasmid (h): sc-44163-SH as alternate gene silencing products.

APPLICATIONS

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

PROTOCOLS

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

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

transgelin (C-11): sc-271719 is recommended as a control antibody for monitoring of transgelin 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 transgelin gene expression knockdown using RT-PCR Primer: transgelin (h)-PR: sc-44163-PR (20 μ l, 530 bp). 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.

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.