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# Calponin 1/2/3 shRNA (h) Lentiviral Particles: sc-43657-V

## BACKGROUND

Calponin regulates smooth muscle cell contraction and is a marker of smooth muscle cell differentiation. Calponin, an Actin- and Tropomyosin-binding protein, is characterized as an inhibitory factor of smooth-muscle actomyosin activity. Calponin is implicated in the regulation of smooth muscle contraction through its interaction with F-Actin and inhibition of the Actin-activated MgATPase activity of phosphorylated myosin. Both properties are lost following phosphorylation (primarily at Serine 175) by protein kinase C or calmodulin-dependent protein kinase II. The three forms of Calponin, Calponin 1 (basic Calponin), Calponin 2 (neutral Calponin) and Calponin 3 (acidic Calponin) are found in smooth muscle tissue. Additionally, Calponin 2 is found in heart muscle tissue and Calponin 3 is found in the brain.

## REFERENCES

1. Tang, D.C., et al. 1996. Structure-function relations of smooth muscle Calponin. The critical role of Serine 175. *J. Biol. Chem.* 271: 8605-8611.
2. Masuda, H., et al. 1996. Molecular cloning and characterization of human non-smooth muscle Calponin. *J. Biochem.* 120: 415-424.
3. Doi, M., et al. 1997. Reduced expression of Calponin in canine basilar artery after subarachnoid haemorrhage. *Acta Neurochir.* 139: 77-81.
4. Kaneko, T., et al. 2000. Identification of Calponin as a novel substrate of Rho-kinase. *Biochem. Biophys. Res. Commun.* 273: 110-116.
5. Yoshimoto, R., et al. 2000. Proteolysis of acidic Calponin by  $\mu$ -calpain. *J. Biochem.* 128: 1045-1049.

## PRODUCT

Calponin 1/2/3 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  $\mu$ l frozen stock containing  $1.0 \times 10^6$  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 Calponin 1/2/3 siRNA (h): sc-43657 and Calponin 1/2/3 shRNA Plasmid (h): sc-43657-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.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog for detailed protocols and support products.

## APPLICATIONS

Calponin 1/2/3 shRNA (h) Lentiviral Particles is recommended for the inhibition of Calponin 1, Calponin 2 and Calponin 3 expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  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

Calponin 1/2/3 (FL-297): sc-28545 is recommended as a control antibody for monitoring of Calponin 1, Calponin 2 and Calponin 3 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immuno-fluorescence (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 Calponin 1, Calponin 2 and Calponin 3 gene expression knockdown using RT-PCR Primer: Calponin 1/2/3 (h)-PR: sc-43657-PR (20  $\mu$ 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.