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Calretinin shRNA (h) Lentiviral Particles: sc-43347-V

BACKGROUND

Calbindin D28K and Calretinin (also designated CR or 29 kDa Calbindin) are two closely related intracellular calcium-binding proteins belonging to the Troponin-C superfamily. Initially isolated from chick retina, Calretinin shares 58% identical residues with human Calbindin D28K. Calretinin is expressed in the brain and is particularly abundant in auditory neurons with precisely timed discharges. Neurons in the nucleus accumbens containing Calretinin all possess nuclear indentations. Calretinin-immunoreactive boutons form asymmetrical and symmetrical synaptic specializations on spines, dendrites and somata. The symmetrical synaptic specializations have medium-sized spiny neurons and contact other Calretinin-immunoreactive somata. Calretinin is widely used as a immunocytochemical marker for mesothelioma.

REFERENCES

1. Rogers, J.H. 1987. Calretinin: a gene for a novel calcium-binding protein expressed principally in neurons. *J. Cell Biol.* 105: 1343-1353.
2. Parmentier, M., et al. 1991. Structure of the human brain calcium-binding protein calretinin and its expression in bacteria. *Eur. J. Biochem.* 196: 79-85.
3. Dreher, B., et al. 1996. Spatiotemporal pattern of ontogenetic expression of calbindin-28/kD in the retinorecipient layers of rat superior colliculus. *J. Comp. Neurol.* 376: 223-240.
4. Hussain, Z., et al. 1996. A light and electron microscopic study of NADPH-diaphorase-, calretinin- and parvalbumin-containing neurons in the rat nucleus accumbens. *J. Chem. Neuroanat.* 10: 19-39.
5. Doglioni, C., et al. 1996. Calretinin: a novel immunocytochemical marker for mesothelioma. *Am. J. Surg. Pathol.* 20: 1037-1046.

CHROMOSOMAL LOCATION

Genetic locus: CALB2 (human) mapping to 16q22.2.

PRODUCT

Calretinin 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 Calretinin siRNA (h): sc-43347 and Calretinin shRNA Plasmid (h): sc-43347-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

Calretinin shRNA (h) Lentiviral Particles is recommended for the inhibition of Calretinin 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

Calretinin (D-12): sc-365989 is recommended as a control antibody for monitoring of Calretinin 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 Calretinin gene expression knockdown using RT-PCR Primer: Calretinin (h)-PR: sc-43347-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.