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parvalbumin α shRNA (h) Lentiviral Particles: sc-43350-V

BACKGROUND

The family of EF-hand type Ca^{2+} -binding proteins includes calbindin (previously designated vitamin D-dependent Ca^{2+} -binding protein), S-100 α and β , calgranulins A (also designated MRP8), B (also designated MRP14) and C (S-100 like proteins) and the parvalbumin family members, including parvalbumin α and parvalbumin β , also designated oncomodulin (OCM). Structurally and evolutionarily conserved, parvalbumin α and OCM proteins are distinct in expression and function. Parvalbumin α , also designated parvalbumin (PV), is most abundantly expressed in fast-contracting muscles with lower expression levels in brain and some endocrine tissues, including kidney and parathyroid. Research indicates that parvalbumin α plays a significant role in muscle relaxation. OCM was originally thought to have expression restricted to neoplastic tissues, early embryonic cells and certain tumor cell lines. Recent research shows that OCM is also expressed and secreted by macrophages where, in the retina it binds to retinal ganglion cells (RGCs) and functions to promote axon regeneration. OCM has also been detected in the auditory sensory cells of the organ of Corti in mammals. In humans, two different loci on chromosome 7 have been identified as OCM and OCM-like (LOC4951). These genes encode proteins 109 amino acids in length which share 99% sequence identity.

REFERENCES

1. Pfyffer, G.E., et al. 1987. Developmental and functional studies of parvalbumin and calbindin D28K in hypothalamic neurons grown in serum-free medium. *J. Neurochem.* 49: 442-451.
2. Kagi, U., et al. 1988. Developmental appearance of the Ca^{2+} -binding proteins parvalbumin, calbindin D-28K, S-100 proteins and calmodulin during testicular development in the rat. *Cell Tissue Res.* 252: 359-365.
3. Muntener, M., et al. 1995. Increase of skeletal muscle relaxation speed by direct injection of parvalbumin cDNA. *Proc. Natl. Acad. Sci. USA* 92: 6504-6508.
4. Cox, J.A., et al. 1999. Remodeling of the AB site of rat parvalbumin and oncomodulin into a canonical EF-hand. *Eur. J. Biochem.* 264: 790-799.

CHROMOSOMAL LOCATION

Genetic locus: PVALB (human) mapping to 22q12.3.

PRODUCT

parvalbumin α 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 parvalbumin α siRNA (h): sc-43350 and parvalbumin α shRNA Plasmid (h): sc-43350-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.

APPLICATIONS

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

parvalbumin α (C-19): sc-7449 is recommended as a control antibody for monitoring of parvalbumin α 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor parvalbumin α gene expression knockdown using RT-PCR Primer: parvalbumin α (h)-PR: sc-43350-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ 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.

PROTOCOLS

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