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Calbindin D9K shRNA (h) Lentiviral Particles: sc-43654-V

BACKGROUND

The family of EF-hand type Ca²⁺-binding proteins includes Calbindin D28K (previously designated vitamin D-dependent Ca²⁺-binding protein), Calbindin D9K, S-100 α and β , Calgranulin A (also designated MRP8), Calgranulin B (also designated MRP14) and Calgranulin C, and the Parvalbumin family members, including Parvalbumin α and Parvalbumin β (also designated oncomodulin). The gene encoding human Calbindin D9K is located on the X chromosome and consists of three exons and contains four Alu repeats. Calbindin D9K protein is present in cartilage, bone and certain teeth, such as the ameloblasts of incisors and molars. In addition, Calbindin D9K mRNA is detected in proximal small intestine, but not in human kidney, uterus or placenta (however, the protein is present in these tissues in other species). Rat Calbindin D9K binds the estrogen receptor because the gene encoding it contains an estrogen response element downstream from its promoter. In contrast, the homologous human sequence differs by two essential nucleotides and does not bind the estrogen receptor, suggesting that this change suppresses gene expression in human tissues, such as uterus and possibly placenta.

REFERENCES

1. Bruns, M.E., et al. 1977. Control of vitamin D-dependent calcium-binding protein in rat intestine by growth and fasting. *J. Biol. Chem.* 252: 4145-4150.
2. Bruns, M.E., et al. 1978. Placental calcium binding protein in rats. Apparent identity with vitamin D-dependent calcium binding protein from rat intestine. *J. Biol. Chem.* 253: 3186-3190.
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4. Delorme, A.C., et al. 1979. Vitamin D-dependent calcium-binding protein. Changes during gestation, prenatal and postnatal development in rats. *J. Dev. Physiol.* 3: 181-194.
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CHROMOSOMAL LOCATION

Genetic locus: S100G (human) mapping to Xp22.2.

PRODUCT

Calbindin D9K 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 Calbindin D9K siRNA (h): sc-43654 and Calbindin D9K shRNA Plasmid (h): sc-43654-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

Calbindin D9K shRNA (h) Lentiviral Particles is recommended for the inhibition of Calbindin D9K 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

Calbindin D9K (D-5): sc-74462 is recommended as a control antibody for monitoring of Calbindin D9K 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 Calbindin D9K gene expression knockdown using RT-PCR Primer: Calbindin D9K (h)-PR: sc-43654-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.

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.

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