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T1R3 shRNA (m) Lentiviral Particles: sc-45325-V

BACKGROUND

The sense of taste provides animals with valuable information about the quality and nutritional value of food. There are four widely accepted categories of taste perception: sweet, bitter, salty and sour. A controversial fifth taste, known as umami or monosodium glutamate (MSG), has also been described. A family of G protein-coupled receptors are involved in taste perception and include T1R, which is involved in sweet and umami taste perception, and T2R, which is involved in bitter taste perception. The T1R family consists of three members: T1R1, T1R2 and T1R3. These proteins form heterodimers, which alter the selectivity of the subunits. The T1R2 and T1R3 heterodimer functions as a receptor for sweet taste, and recognizes several sweet-tasting molecules such as sucrose, saccharin, dulcin and acesulfame-K. The T1R1 and T1R3 heterodimer recognizes L-amino acids to perceive umami taste. Sweet taste transduction is carried out by two pathways. First, sucrose and other sugars activate Gas via the T1Rs, which activates adenylyl cyclase to generate cAMP. Artificial sweeteners bind to either $G_{\beta\gamma}$ - or $G_{\alpha q}$ -coupled T1Rs to activate PLC β 2 and generate IP3 and DAG. Both pathways ultimately lead to neurotransmitter release. The mouse T1R3 gene maps to chromosome 4 near the Sac locus, a primary determinant of sweet preference in mice. It is expressed in a subset of taste cells in circumvallate, foliate and fungiform taste papillae.

REFERENCES

1. Nelson, G., et al. 2001. Mammalian sweet taste receptors. *Cell* 106: 381-390.
2. Montmayeur, J.P., et al. 2001. A candidate taste receptor gene near a sweet taste locus. *Nat. Neurosci.* 4: 492-498.
3. Sainz, E., et al. 2001. Identification of a novel member of the T1R family of putative taste receptors. *J. Neurochem.* 77: 896-903.

CHROMOSOMAL LOCATION

Genetic locus: Tas1r3 (mouse) mapping to 4 E2.

PRODUCT

T1R3 shRNA (m) 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 T1R3 siRNA (m): sc-45325 and T1R3 shRNA Plasmid (m): sc-45325-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

T1R3 shRNA (m) Lentiviral Particles is recommended for the inhibition of T1R3 expression in mouse 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

T1R3 (G-2): sc-398996 is recommended as a control antibody for monitoring of T1R3 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 T1R3 gene expression knockdown using RT-PCR Primer: T1R3 (m)-PR: sc-45325-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.