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CRB3 shRNA (h) Lentiviral Particles: sc-43698-V

BACKGROUND

The transmembrane protein Crumbs plays a crucial role in epithelial cell polarity and photoreceptor development in *Drosophila melanogaster* embryos, but the first identified human homologue, CRB1, is only expressed in retina and some parts of the brain, leaving room for another homologue to function in epithelial tissues. Leber congenital amaurosis or progressive retinitis pigmentosa are caused by loss of CRB1 function. A second homologue, CRB3, fills the gap, showing expression in epithelial tissues as well as skeletal muscles. CRB3 shares a cytoplasmic domain with other Crumbs proteins, but contains only a very short extracellular domain, through which it interacts with Par6, a regulator of epithelial polarity and tight junction formation. Thus, this specialized isoform provides a connection between apical membrane formation and tight junction regulation.

REFERENCES

1. Roh, M.H., et al. 2002. The Maguk protein, Pals1, functions as an adapter, linking mammalian homologues of Crumbs and Discs Lost. *J. Cell Biol.* 157: 161-172.
2. Makarova, O., et al. 2003. Mammalian Crumbs3 is a small transmembrane protein linked to protein associated with Lin-7 (Pals1). *Gene* 302: 21-29.
3. van de Pavert, S.A., et al. 2004. Crumbs homologue 1 is required for maintenance of photoreceptor cell polarization and adhesion during light exposure. *J. Cell Sci.* 117: 4169-4177.
4. Lemmers C, Michel, D., et al. 2004. CRB3 binds directly to Par6 and regulates the morphogenesis of the tight junctions in mammalian epithelial cells. *Mol. Biol. Cell* 15: 1324-1333.

CHROMOSOMAL LOCATION

Genetic locus: CRB3 (human) mapping to 19p13.3.

PRODUCT

CRB3 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 CRB3 siRNA (h): sc-43698 and CRB3 shRNA Plasmid (h): sc-43698-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.

APPLICATIONS

CRB3 shRNA (h) Lentiviral Particles is recommended for the inhibition of CRB3 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

CRB3 (K-20): sc-27903 is recommended as a control antibody for monitoring of CRB3 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 CRB3 gene expression knockdown using RT-PCR Primer: CRB3 (h)-PR: sc-43698-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.

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.