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TRB-3 shRNA (m) Lentiviral Particles: sc-44427-V

BACKGROUND

TRB-3 (tribbles 3), also called NIPK (neuronal cell death-inducible protein kinase) disrupts Insulin signaling by binding directly to Akt kinases and blocking their activation. TRB-3 binds to ATF4, inhibiting its transcriptional activation activity, and regulates activation of MAP kinases. In the liver, TRB-3 is a target for PPAR α . Amounts of TRB-3 RNA and protein are higher in livers of diabetic mice compared with those in wildtype mice. TRB-3 contributes to Insulin resistance in individuals with susceptibility to type II diabetes. Highest expression of TRB-3 is in liver, pancreas, peripheral blood leukocytes and bone marrow.

REFERENCES

1. Du, K., et al. 2003. TRB-3: a tribbles homolog that inhibits Akt/PKB activation by Insulin in liver. *Science* 300: 1574-1577.
2. Kiss-Toth, E., et al. 2004. Human tribbles, a protein family controlling mitogen-activated protein kinase cascades. *J. Biol. Chem.* 279: 42703-42708.
3. Koo, S.H., et al. 2004. PGC-1 promotes Insulin resistance in liver through PPAR α -dependent induction of TRB-3. *Nat. Med.* 10: 530-534.
4. Ohoka, N., et al. 2005. TRB-3, a novel ER stress-inducible gene, is induced via ATF4-CHOP pathway and is involved in cell death. *EMBO J.* 24: 1243-1255.
5. Ord, D., et al. 2005. Characterization of human NIPK (TRB-3, SKIP3) gene activation in stressful conditions. *Biochem. Biophys. Res. Commun.* 330: 210-218.
6. Prudente, S., et al. 2005. The functional Q84R polymorphism of mammalian tribbles homolog TRB-3 is associated with Insulin resistance and related cardiovascular risk in caucasians from Italy. *Diabetes* 54: 2807-2811.

CHROMOSOMAL LOCATION

Genetic locus: Trib3 (mouse) mapping to 2 G3.

PRODUCT

TRB-3 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 2 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 TRB-3 siRNA (m): sc-44427 and TRB-3 shRNA Plasmid (m): sc-44427-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

TRB-3 shRNA (m) Lentiviral Particles is recommended for the inhibition of TRB-3 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

TRB-3 (B-2): sc-390242 is recommended as a control antibody for monitoring of TRB-3 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 TRB-3 gene expression knockdown using RT-PCR Primer: TRB-3 (m)-PR: sc-44427-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.