

TRB-3 siRNA (m): sc-44427

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. TRB3 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 TRB3 RNA and protein are higher in livers of diabetic mice compared with those in wildtype mice. TRB3 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. TRB3, 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 (TRB3, SKIP3) gene activation in stressful conditions. *Biochem. Biophys. Res. Commun.* 330: 210-218.

CHROMOSOMAL LOCATION

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

PRODUCT

TRB-3 siRNA (m) is a pool of 2 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TRB-3 shRNA Plasmid (m): sc-44427-SH and TRB-3 shRNA (m) Lentiviral Particles: sc-44427-V as alternate gene silencing products.

For independent verification of TRB-3 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44427A and sc-44427B.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

TRB-3 siRNA (m) is recommended for the inhibition of TRB-3 expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

TRB-3 (D-4): sc-365842 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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

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° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Fan, J., et al. 2015. Delivery of phenamil enhances BMP-2-induced osteogenic differentiation of adipose-derived stem cells and bone formation in calvarial defects. *Tissue Eng. Part A* 21: 2053-2065.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.