SANTA CRUZ BIOTECHNOLOGY, INC.

TOB2 siRNA (m): sc-37507

BACKGROUND

TOB1 (TROB, APRO6, PIG49) and TOB2 (TOB4, TROB2, TOBL) are anti-proliferative proteins that modulate cell cycle progression from the G_0/G_1 to S phases through interactions with the mammalian homologue of yeast Caf1. TOB proteins present in the central nervous system may be engaged in acquisition of motor skill. TOB1 in T lymphocytes can interact with Smad2/4, augment SMAD DNA binding to the IL-2 promoter, and lead to an inhibition of IL-2 transcription. In oncogenic ErbB-2-transformed cells, nuclear export of TOB1 results in a decrease in antiproliferative activity. ERK/MAPK (ERK2) and JNK/SAPK (JNK2) phosphorylate TOB1 *in vitro,* and TOB1 can undergo phosphorylation at Ser 152, Ser 154 and Ser 164 by ERK1/2 upon growth-factor stimulation. TOB2 gene encodes a 4.1-kb transcript with high expression in skeletal muscle, thymus and ovary.

REFERENCES

1. Matsuda, S., et al. 1996. TOB, a novel protein that interacts with p185erbB2, is associated with anti-proliferative activity. Oncogene 12: 705-713.
2. Ikematsu, N., et al. 1999. TOB2, a novel antiproliferative TOB/BTG1 family member, associates with a component of the CCR4 transcriptional regulatory complex capable of binding cyclin-dependent kinases. Oncogene 18: 7432-7441.
3. Ajima, R., et al. 2000. Cloning and characterization of the mouse TOB2 gene. Gene 253: 215-220.
4. Yoshida, Y., et al. 2000. Negative regulation of BMP/Smad signaling by TOB in osteoblasts. Cell 103: 1085-1097.
5. Tzachanis, D., et al. 2001. TOB is a negative regulator of activation that is expressed in anergic and quiescent T cells. Nat. Immunol. 2: 1174-1182.
6. Suzuki, T., et al. 2002. Phosphorylation of three regulatory serines of TOB by ERK 1 and ERK 2 is required for Ras-mediated cell proliferation and transformation. Genes Dev. 16: 1356-1370.
7. Maekawa, M., et al. 2002. Identification of the anti-proliferative protein TOB as a MAPK substrate. J. Biol. Chem. 277: 37783-37787.
8. Kawamura-Tsuzuku, J., et al. 2004. Nuclear localization of TOB is important for regulation of its antiproliferative activity. Oncogene 23: 6630-6638.

CHROMOSOMAL LOCATION

Genetic locus: Tob2 (mouse) mapping to 15 E1.

PRODUCT

TOB2 siRNA (m) is a pool of 3 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TOB2 shRNA Plasmid (m): sc-37507-SH and TOB2 shRNA (m) Lentiviral Particles: sc-37507-V as alternate gene silencing products.

For independent verification of TOB2 (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-37507A, sc-37507B and sc-37507C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

TOB2 siRNA (m) is recommended for the inhibition of TOB2 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 µM in 66 µl. 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

TOB2 (B-4): sc-515829 is recommended as a control antibody for monitoring of TOB2 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 TOB2 gene expression knockdown using RT-PCR Primer: TOB2 (m)-PR: sc-37507-PR (20 µl, 461 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.