SignalSilence® TRIM33 siRNA I

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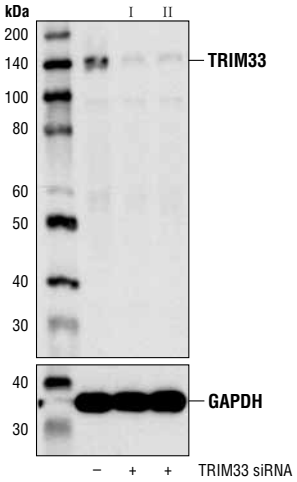
For Research Use Only. Not For Use In Diagnostic Procedures.

Species Cross-Reactivity: H

Description: SignalSilence® TRIM33 siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit TRIM33 expression using RNA interference, a method whereby gene expression can be selectively silenced through the delivery of double stranded RNA molecules into the cell. All SignalSilence® siRNA products from CST are rigorously tested in-house and have been shown to reduce target protein expression by western analysis.

Background: The transcriptional intermediary factor 1 (TIF1) family represents a group of proteins with multiple histone-binding domains. In humans, this family comprises four proteins, TIF1α/TRIM24, TIF1β/TRIM28/KAP1, TIF1γ/TRIM33/Ectodermin, and TIF1δ/TRIM66, which are characterized by an amino-terminal tripartite motif (TRIM) domain consisting of a RING domain, two B boxes, a coiled-coil domain, and a carboxy-terminal PHD finger and bromodomain (1). Despite their similar overall structure, these proteins have diverse roles in transcriptional regulation. TIF1α functions as a ligand-dependent nuclear receptor coregulator and more recently has been implicated in regulating p53 stability (2). TIF1β is an intrinsic component of the N-CoR1 corepressor complex and the NuRD nucleosome-remodeling complex (3) and functions as a corepressor for Kruppel-associated box (KRAB) zinc-finger transcription factors (4). Furthermore, TIF1β promotes heterochromatin-mediated gene silencing formation by serving as a cofactor for heterochromatin protein HP1 (5). TIF1δ expression is restricted to the testis and has been shown to interact with HP1γ (6).

In contrast, the ubiquitous nuclear protein TRIM33 does not interact with either HP1 family members or chromatin-remodeling/modifying complexes. Rather, TRIM33 plays a pivotal role in signaling cascades driven by the TGF-β superfamily of ligands (7-9). A research study suggests that TRIM33 and Smad4 compete for binding to receptor phosphorylated Smad2/3 and that TRIM33-Smad2/3 and Smad4-Smad2/3 complexes complement one another in the TGF-β-dependent control of hematopoietic cell fate (9). Other studies, however, demonstrate that TRIM33 functions to repress signal relay by the TGF-β superfamily (7-8,10). Indeed, knockout of murine *Trim33* results in embryonic lethality due to upregulated Nodal signaling (10). Mechanistically, TRIM33 functions as an E3-ubiquitin ligase and promotes monoubiquitination of Smad4, a modification that impairs its ability to associate with phospho-Smad2 (8). This negative regulatory mechanism is further substantiated by the discovery that TRIM33 disrupts transcriptionally competent Smad complexes on the promoter/enhancer regions of TGF-β-responsive genes by associating with specific epigenetic marks on histone H3, which is a requirement for activating TRIM33's monoubiquitin ligase activity toward Smad4 (11). In line with the ability of TRIM33 to regulate the development of different blood cell lineages, it was shown that loss of TRIM33 expression due to epigenetic silencing of its promoter contributes to the pathogenesis of chronic myelomonocytic leukemia (12).



Western blot analysis of extracts from 293T cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® TRIM33 siRNA I (+), or SignalSilence® TRIM33 siRNA II #13503 (+), using TRIM33 (E1N2Z) Rabbit mAb #13387 (upper) or GAPDH (D16H11) XP® Rabbit mAb #5174 (lower). The TRIM33 (E1N2Z) Rabbit mAb confirms silencing of TRIM33 expression, while the GAPDH (D16H11) XP® Rabbit mAb is used as a loading control.

Directions for Use: CST recommends transfection with 100 nM SignalSilence® TRIM33 siRNA I 48 to 72 hours prior to cell lysis. For transfection procedure, follow protocol provided by the transfection reagent manufacturer. Please feel free to contact CST with any questions on use.

Each vial contains the equivalent of 100 transfections, which corresponds to a final siRNA concentration of 100 nM per transfection in a 24-well plate with a total volume of 300 µl per well.

Quality Control: Oligonucleotide synthesis is monitored base by base through trityl analysis to ensure appropriate coupling efficiency. The oligo is subsequently purified by affinity-solid phase extraction. The annealed RNA duplex is further analyzed by mass spectrometry to verify the exact composition of the duplex. Each lot is compared to the previous lot by mass spectrometry to ensure maximum lot-to-lot consistency.

Entrez-Gene ID #51592
UniProt Acc. #Q9UPN9

Storage: TRIM33 siRNA I is supplied in RNAse-free water. *Aliquot and store at -20°C.*

For product specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

(1) Meroni, G. and Diez-Roux, G. (2005) *Bioessays* 27, 1147-57.
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(3) Underhill, C. et al. (2000) *J Biol Chem* 275, 40463-70.
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(5) Groner, A.C. et al. (2010) *PLoS Genet* 6, e1000869.
(6) Khetchoumian, K. et al. (2004) *J Biol Chem* 279, 48329-41.
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(12) Aucagne, R. et al. (2011) *J Clin Invest* 121, 2361-70.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.