SANTA CRUZ BIOTECHNOLOGY, INC.

TTI1 siRNA (h): sc-75381

BACKGROUND

TTI1 (TELO2-interacting protein 1 homolog), also known as SMG10, is a 1,089 amino acid protein that is widely expressed and belongs to the TTI1 family. TTI1 functions as a regulator of the DNA damage response (DDR) and is a component of the TTT complex, which is necessary for the stabilization of protein levels of the phosphatidylinositol 3-kinase (PIKK) family. The TTT complex is a part of the cellular resistance to DNA damage stresses such as ionizing radiation (IR), ultraviolet (UV) and mitomycin C (MMC). In combination with the TTT complex and HSP 90, TTI1 may play a role in the proper folding of newly synthesized PIKKs. TTI1 also is involved in the assembly of mTORC1 and mTORC2 complexes, as well as their stabilization and maintenance. TTI1 is post-tanslationally modified at serine 459 and the gene encoding this protein maps to human chromosome 20q11.23.

REFERENCES

1. Ishikawa, K., et al. 1997. Prediction of the coding sequences of unidentified human genes. VIII. 78 new cDNA clones from brain which code for large proteins *in vitro*. DNA Res. 4: 307-313.
2. Olsen, J.V., et al. 2006. Global, *in vivo,* and site-specific phosphorylation dynamics in signaling networks. Cell 127: 635-648.
3. Daub, H., et al. 2008. Kinase-selective enrichment enables quantitative phosphoproteomics of the kinome across the cell cycle. Mol. Cell 31: 438-448.
4. Hurov, K.E., et al. 2010. A genetic screen identifies the Triple T complex required for DNA damage signaling and ATM and ATR stability. Genes Dev. 24: 1939-1950.
5. Takai, H., et al. 2010. Tel2 structure and function in the Hsp90-dependent maturation of mTOR and ATR complexes. Genes Dev. 24: 2019-2030.
6. Kaizuka, T., et al. 2010. TTI1 and Tel2 are critical factors in mammalian target of rapamycin complex assembly. J. Biol. Chem. 285: 20109-20116.
7. Izumi, N., et al. 2010. AAA$^+$ proteins RUVBL1 and RUVBL2 coordinate PIKK activity and function in nonsense-mediated mRNA decay. Sci. Signal. 3: ra27.

CHROMOSOMAL LOCATION

Genetic locus: TTI1 (human) mapping to 20q11.23.

PRODUCT

TTI1 siRNA (h) 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 TTI1 shRNA Plasmid (h): sc-75381-SH and TTI1 shRNA (h) Lentiviral Particles: sc-75381-V as alternate gene silencing products.

For independent verification of TTI1 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-75381A, sc-75381B and sc-75381C.

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

TTI1 siRNA (h) is recommended for the inhibition of TTI1 expression in human 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

TTI1 (B-1): sc-365119 is recommended as a control antibody for monitoring of TTI1 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 TTI1 gene expression knockdown using RT-PCR Primer: TTI1 (h)-PR: sc-75381-PR (20 µl). 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.