

Tomosyn siRNA (h): sc-76713

BACKGROUND

WD-repeats are motifs that are found in a variety of proteins and are characterized by a conserved core of 40-60 amino acids that commonly form a tertiary propeller structure. While proteins that contain WD-repeats participate in a wide range of cellular functions, they are generally involved in regulatory mechanisms concerning chromatin assembly, cell cycle control, signal transduction, RNA processing, apoptosis and vesicular trafficking. Tomosyn, also known as STXBP5 (syntaxin binding protein 5), LLGL3 or LGL3, is a 1,151 amino acid protein that localizes to the cytoplasm, as well as to the cell junction, secretory vesicles and to the peripheral membrane and contains one v-SNARE coiled-coil homology domain and 14 WD repeats. Interacting with Syntaxin 1 and Syntaxin 1B, Tomosyn functions as a regulator of neurotransmitter release and calcium-dependent exocytosis. Additionally, Tomosyn inhibits membrane fusion and may play a role in the assembly of SNARE complexes between transport vesicles and the plasma membrane. Multiple isoforms of Tomosyn exist due to alternative splicing events.

REFERENCES

1. van der Voorn, L., et al. 1992. The WD-40 repeat. *FEBS Lett.* 307: 131-134.
2. Fujita, Y., et al. 1998. Tomosyn: a syntaxin-1-binding protein that forms a novel complex in the neurotransmitter release process. *Neuron* 20: 905-915.
3. Yokoyama, S., et al. 1999. Three splicing variants of tomosyn and identification of their syntaxin-binding region. *Biochem. Biophys. Res. Commun.* 256: 218-222.
4. Smith, T.F., et al. 1999. The WD repeat: a common architecture for diverse functions. *Trends Biochem. Sci.* 24: 181-185.
5. Widberg, C.H., et al. 2003. Tomosyn interacts with the t-SNAREs syntaxin4 and SNAP23 and plays a role in Insulin-stimulated GLUT4 translocation. *J. Biol. Chem.* 278: 35093-35101.
6. Katoh, M., et al. 2004. Identification and characterization of human LLGL4 gene and mouse Lgl4 gene in silico. *Int. J. Oncol.* 24: 737-742.
7. Yizhar, O., et al. 2004. Tomosyn inhibits priming of large dense-core vesicles in a calcium-dependent manner. *Proc. Natl. Acad. Sci. USA* 101: 2578-2583.

CHROMOSOMAL LOCATION

Genetic locus: STXBP5 (human) mapping to 6q24.3.

PRODUCT

Tomosyn 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Tomosyn shRNA Plasmid (h): sc-76713-SH and Tomosyn shRNA (h) Lentiviral Particles: sc-76713-V as alternate gene silencing products.

For independent verification of Tomosyn (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-76713A, sc-76713B and sc-76713C.

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

Tomosyn siRNA (h) is recommended for the inhibition of Tomosyn 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 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

Tomosyn (15): sc-136105 is recommended as a control antibody for monitoring of Tomosyn 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 Tomosyn gene expression knockdown using RT-PCR Primer: Tomosyn (h)-PR: sc-76713-PR (20 μ l). 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.