SANTA CRUZ BIOTECHNOLOGY, INC.

Tom1L-1 siRNA (h): sc-76708

BACKGROUND

Tom1L-1 (target of myb1 (chicken)-like 1), also known as SRCASM or OK/KNS-CL.3, is a 476 amino acid Golgi apparatus protein belonging to the TOM1 family and is a member of the multivesicular body (MVB) sorting machinery. Containing a GAT domain and a VHS domain, Tom1L-1 interacts with Fyn, GRB2, PI 3-kinase p85α and various signaling proteins when phosphorylated. GAT domain of Tom1L1 binds ubiquitin, suggesting participation in the sorting of ubiquitinated proteins into MVBs. Tom1L-1 may act as an adapter protein involved in signaling pathways and may promote Fyn activation, possibly by disrupting intramolecular SH3-dependent interactions. As an interactor and a substrate of Src tyrosine kinases (SFK), Tom1L1 is considered a novel mechanism involved in negative regulation of SFK mitogenic and transforming signals. Tom1L1 modulates SFK partitioning at the plasma membrane and downregulates Src kinases in an endosomal-dependent manner. It is suggested that Tom1L-1 functions as an anti-oncogene by inhibiting the formation of squamous cell carcinomas in skin.

REFERENCES

1. Seykora, J.T., et al. 2002. Srcasm: a novel Src activating and signaling molecule. J. Biol. Chem. 277: 2812-2822.
2. Puertollano, R. 2005. Interactions of TOM1L1 with the multivesicular body sorting machinery. J. Biol. Chem. 280: 9258-9264.
3. Franco, M., et al. 2006. The adaptor protein Tom1L1 is a negative regulator of Src mitogenic signaling induced by growth factors. Mol. Cell. Biol. 26: 1932-1947.
4. Li, W., et al. 2007. Srcasm corrects Fyn-induced epidermal hyperplasia by kinase down-regulation. J. Biol. Chem. 282: 1161-1169.
5. Collin, G., et al. 2007. The Tom1L1-clathrin heavy chain complex regulates membrane partitioning of the tyrosine kinase Src required for mitogenic and transforming activities. Mol. Cell. Biol. 27: 7631-7640.
6. Emaduddin, M., et al. 2008. Odin (ANKS1A) is a Src family kinase target in colorectal cancer cells. Cell Commun. Signal. 6: 7.

CHROMOSOMAL LOCATION

Genetic locus: TOM1L1 (human) mapping to 17q22.

PRODUCT

Tom1L-1 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 Tom1L-1 shRNA Plasmid (h): sc-76708-SH and Tom1L-1 shRNA (h) Lentiviral Particles: sc-76708-V as alternate gene silencing products.

For independent verification of Tom1L-1 (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-76708A, sc-76708B and sc-76708C.

RESEARCH USE

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

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

Tom1L-1 siRNA (h) is recommended for the inhibition of Tom1L-1 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

Tom1L-1 (3F12): sc-130565 is recommended as a control antibody for monitoring of Tom1L-1 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Tom1L-1 gene expression knockdown using RT-PCR Primer: Tom1L-1 (h)-PR: sc-76708-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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