

LRSAM1 siRNA (h): sc-92826

BACKGROUND

Ubiquitin is an abundant, highly conserved protein found in all eukaryotic cells either free or covalently attached to cellular proteins. Ubiquitination is an important mechanism through which three classes of enzymes act in concert to target short-lived or abnormal proteins for destruction. The three classes of enzymes involved in ubiquitination are the ubiquitin-activating enzymes (E1s), the ubiquitin-conjugating enzymes (E2s) and the ubiquitin-protein ligases (E3s). LRSAM1 (leucine-rich repeat and sterile α motif-containing protein 1), also known as Tsg101-associated ligase, is a 723 amino acid E3 ubiquitin-protein ligase that mediates the monoubiquitination of tsg 101, a regulator of vesicular trafficking. This leads to inactivation of the sorting of endocytic and exocytic cargos, such as EGF receptors and HIV-1 viral proteins. Mutations within the LRSAM1 gene have been identified in patients with Charcot-Marie-Tooth disease. There are three isoforms of LRSAM1 that are produced as a result of alternative splicing events.

REFERENCES

1. Amit, I., et al. 2004. Tal, a Tsg101-specific E3 ubiquitin ligase, regulates receptor endocytosis and retrovirus budding. *Genes Dev.* 18: 1737-1752.
2. Carlton, J.G., et al. 2007. Parallels between cytokinesis and retroviral budding: a role for the ESCRT machinery. *Science* 316: 1908-1912.
3. McDonald, B., et al. 2008. Regulation of Tsg101 expression by the steadiness box: a role of Tsg101-associated ligase. *Mol. Biol. Cell* 19: 754-763.
4. Guernsey, D.L., et al. 2010. Mutation in the gene encoding ubiquitin ligase LRSAM1 in patients with Charcot-Marie-Tooth disease. *PLoS Genet.* 6: e1001081.
5. Ng, A.C., et al. 2010. Human leucine-rich repeat proteins: a genome-wide bioinformatic categorization and functional analysis in innate immunity. *Proc. Natl. Acad. Sci. USA* 108: 4631-4638.

CHROMOSOMAL LOCATION

Genetic locus: LRSAM1 (human) mapping to 9q33.3.

PRODUCT

LRSAM1 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 LRSAM1 shRNA Plasmid (h): sc-92826-SH and LRSAM1 shRNA (h) Lentiviral Particles: sc-92826-V as alternate gene silencing products.

For independent verification of LRSAM1 (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-92826A, sc-92826B and sc-92826C.

PROTOCOLS

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

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

LRSAM1 siRNA (h) is recommended for the inhibition of LRSAM1 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor LRSAM1 gene expression knockdown using RT-PCR Primer: LRSAM1 (h)-PR: sc-92826-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Mishra, R., et al. 2020. LRSAM1 E3 ubiquitin ligase promotes proteasomal clearance of E6-AP protein. *Cell. Signal.* 77: 109836.

RESEARCH USE

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