SANTA CRUZ BIOTECHNOLOGY, INC.

Slp5 siRNA (h): sc-91313

BACKGROUND

Synaptotagmin genes encode a large family of synaptic vesicle type III integral membrane proteins that function as regulators of both exocytosis and endocytosis and are involved in neurotransmitter secretion from small secretory vesicles. Slp5 (synaptotagmin-like protein 5), also known as SYTL5, is a 730 amino acid peripheral membrane protein that contains one Rab binding domain, one FYVE-type zinc finger and 2 C2 domains. Highly expressed in liver and placenta, Slp5 is thought to act as a Rab effector protein that, similar to synaptotagmins, may play a role in vesicle trafficking. Slp5 preferentially interacts with the GTP-bound form of Rab27a and marginally interacts with Rab3A and Rab6A, but not with other Rab proteins. It is though that Rab27a may play a role in cystic fibrosis pathogenesis by inhibiting CFTR channel activity. Slp5 limits Rab27a availability to CTFR, therefore minimizing its effect on channel function. This suggests that Slp5 may be a potential target for cystic fibrosis therapy.

REFERENCES

1. Kuroda, T.S., et al. 2002. Synaptotagmin-like protein 5: a novel Rab27A effector with C-terminal tandem C2 domains. Biochem. Biophys. Res. Commun. 293: 899-906.
2. Kuroda, T.S., et al. 2002. The Slp homology domain of synaptotagmin-like proteins 1-4 and Slac2 functions as a novel Rab27A binding domain. J. Biol. Chem. 277: 9212-9218.
3. Saxena, S.K., et al. 2006. Rab27a regulates epithelial sodium channel (ENaC) activity through synaptotagmin-like protein (SLP-5) and Munc13-4 effector mechanism. Biochem. Biophys. Res. Commun. 344: 651-657.
4. Saxena, S.K. and Kaur, S. 2006. Rab27a negatively regulates CFTR chloride channel function in colonic epithelia: involvement of the effector proteins in the regulatory mechanism. Biochem. Biophys. Res. Commun. 346: 259-267.
5. Tsuboi, T. and Fukuda, M. 2006. The Slp4-a linker domain controls exocytosis through interaction with Munc18-1.syntaxin-1a complex. Mol. Biol. Cell 17: 2101-2112.
6. Holt, O., et al. 2008. Slp1 and Slp2-a localize to the plasma membrane of CTL and contribute to secretion from the immunological synapse. Traffic 9: 446-457.

CHROMOSOMAL LOCATION

Genetic locus: SYTL5 (human) mapping to Xp11.4.

PRODUCT

Slp5 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 Slp5 shRNA Plasmid (h): sc-91313-SH and Slp5 shRNA (h) Lentiviral Particles: sc-91313-V as alternate gene silencing products.

For independent verification of Slp5 (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-91313A, sc-91313B and sc-91313C.

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

Slp5 siRNA (h) is recommended for the inhibition of Slp5 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

Slp5 (S-12): sc-241673 is recommended as a control antibody for monitoring of Slp5 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Slp5 gene expression knockdown using RT-PCR Primer: Slp5 (h)-PR: sc-91313-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.