

Munc13-3 siRNA (m): sc-42025

BACKGROUND

Munc13 proteins (Munc13-1, Munc13-2, and Munc13-3) make up a family of highly homologous synaptic molecules that bind Syntaxin, an essential mediator of neurotransmitter release. Munc13 proteins contain phorbol ester binding C1- and C2-domains, which are regulatory domains for Ca^{2+} , phospholipids and diacylglycerol. Munc13 proteins are primarily expressed by neurons, except for a ubiquitously expressed Munc13-2 splice variant. Munc13-1 is expressed by most neurons; it interacts with the N-terminal of Doc2 α , which is concentrated on the synaptic vesicle. Munc13-1 also interacts directly with msec7-1 to co-localize the two proteins at the active zone, a presynaptic, subcellular compartment with extremely high membrane turnover. Munc13-1 is essential for synaptic vesicle maturation and plays a role in the central priming function in synaptic vesicle exocytosis from glutamatergic synapses. Munc13-1 is concentrated in presynaptic terminals. Munc13-2 is expressed in rostral regions, whereas Munc13-3 is expressed primarily in the cerebellum.

REFERENCES

1. Brose, N., et al. 1995. Mammalian homologues of *Caenorhabditis elegans* unc-13 gene define novel family of C2-domain proteins. *J. Biol. Chem.* 270: 25273-25280.
2. Mochida, S., et al. 1998. Role of the Doc2 α -Munc13-1 interaction in the neurotransmitter release process. *Proc. Natl. Acad. Sci. USA* 95: 11418-11422.
3. Augustin, I., et al. 1999. Differential expression of two novel Munc13 proteins in rat brain. *Biochem. J.* 337: 363-371.
4. Neeb, A., et al. 1999. Direct interaction between the ARF-specific guanine nucleotide exchange factor msec7-1 and presynaptic Munc13-1. *Eur. J. Cell Biol.* 78: 533-538.
5. Augustin, I., et al. 1999. Munc13-1 is essential for fusion competence of glutamatergic synaptic vesicles. *Nature* 400: 457-461.
6. Koch, H., et al. 2000. Definition of Munc13-homology-domains and characterization of a novel ubiquitously expressed Munc13 isoform. *Biochem. J.* 349: 247-253.

CHROMOSOMAL LOCATION

Genetic locus: Unc13c (mouse) mapping to 9 D.

PRODUCT

Munc13-3 siRNA (m) 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 Munc13-3 shRNA Plasmid (m): sc-42025-SH and Munc13-3 shRNA (m) Lentiviral Particles: sc-42025-V as alternate gene silencing products.

For independent verification of Munc13-3 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-42025A, sc-42025B and sc-42025C.

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

Munc13-3 siRNA (m) is recommended for the inhibition of Munc13-3 expression in mouse 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

Munc13-1/2/3 (32): sc-136182 is recommended as a control antibody for monitoring of Munc13-1 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 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 Munc13-3 gene expression knockdown using RT-PCR Primer: Munc13-3 (m)-PR: sc-42025-PR (20 μl). Annealing temperature for the primers should be $55-60^{\circ}\text{C}$ and the extension temperature should be $68-72^{\circ}\text{C}$.

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.