SANTA CRUZ BIOTECHNOLOGY, INC.

Unc18-3 siRNA (m): sc-42313

BACKGROUND

Unc18-1, -2 and -3 (syntaxin binding proteins 1-3, STXBP1-3, UNC18-a–c, MUNC18-1–3) are chaperone molecules that block syntaxin interactions with cognate SNARE (soluble NSF attachment protein (SNAP) receptors) proteins and regulate exocytosis. Unc18-1–3 mRNA is present in RBL-2H3 mast cells, mouse bone marrow derived mast cells (BMMC) and platelets. Unc18-1 Ser 313 is a protein kinase C phosphorylation site and Thr 574 is a cyclin-dependent kinase 5 phosphorylation site that regulates Unc18-1/Syntaxin 1A interactions. Unc18-1 is phosphorylated on Ser 313 in response to phorbol ester treatment in adrenal chromaffin cells. Unc18-2 co-localizes with Syntaxin 3 at the apical plasma membrane of intestinal, proximal tubule and collecting duct epithelial cells.

REFERENCES

1. Schraw, T.D., et al. 2003. A role for Sec1/Munc18 proteins in platelet exocytosis. Biochem. J. 374: 207-217.
2. Barclay, J.W., et al. 2003. Phosphorylation of Munc18 by protein kinase C regulates the kinetics of exocytosis. J. Biol. Chem. 278: 10538-10545.
3. Gaisano, H.Y., et al. 2004. Alcoholic chronic pancreatitis involves displacement of Munc18c from the pancreatic acinar basal membrane surface. Pancreas 28: 395-400.
4. Graham, M.E., et al. 2004. Syntaxin/Munc18 interactions in the late events during vesicle fusion and release in exocytosis. J. Biol. Chem. 279: 32751-32760.
5. Gladycheva, S.E., et al. 2004. Regulation of Syntaxin 1A-Munc18 complex for SNARE pairing in HEK293 cells. J. Physiol. 558: 857-871.
6. Liu, J., et al. 2004. Fluorescence resonance energy transfer reports properties of Syntaxin 1A interaction with Munc18-1 *in vivo*. J. Biol. Chem. 279: 55924-55936.
7. Ciufo, L.F., et al. 2005. Munc18-1 regulates early and late stages of exocytosis via syntaxin-independent protein interactions. Mol. Biol. Cell 16: 470-482.
8. Nigam, R., et al. 2005. Expression and transcriptional regulation of Munc18 isoforms in mast cells. Biochim. Biophys. Acta 1728: 77-83.

CHROMOSOMAL LOCATION

Genetic locus: Stxbp3a (mouse) mapping to 3 F3.

PRODUCT

Unc18-3 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 Unc18-3 shRNA Plasmid (m): sc-42313-SH and Unc18-3 shRNA (m) Lentiviral Particles: sc-42313-V as alternate gene silencing products.

For independent verification of Unc18-3 (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-42313A, sc-42313B and sc-42313C.

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

Unc18-3 siRNA (m) is recommended for the inhibition of Unc18-3 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

Unc18-3 (H-7): sc-373813 is recommended as a control antibody for monitoring of Unc18-3 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 Unc18-3 gene expression knockdown using RT-PCR Primer: Unc18-3 (m)-PR: sc-42313-PR (20 µl). 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.