

# mAChR M3 siRNA (h): sc-35833

## BACKGROUND

The muscarinic acetylcholine receptors (mAChR) mediate a variety of cellular responses, including inhibition of adenylate cyclase, breakdown of phosphoinositides and modulation of potassium channels. The mAChRs transduce signals by coupling to G proteins, which then modulate several downstream effector proteins and ion channels. Five mAChR subtypes have been identified, designated M1 to M5. The five receptor subtypes show distinct patterns of tissue distribution, as well as distinct pharmacological and functional properties. The amino acid sequence of each mAChR subtype reflects a structure that is characteristic of G protein-coupled receptors, consisting of seven highly conserved transmembrane segments and a large intracellular region unique to each subtype, which constitutes the effector-coupling domain.

## REFERENCES

1. Peralta, E.G., et al. 1987. Primary structure and biochemical properties of an M2 muscarinic receptor. *Science* 236: 600-605.
2. Liao, C.F., et al. 1989. Molecular cloning and expression of a fifth muscarinic acetylcholine receptor. *J. Biol. Chem.* 264: 7328-7337.
3. Hulme, E.C. 1990. Muscarinic acetylcholine receptors: typical G-coupled receptors. *Symp. Soc. Exp. Biol.* 44: 39-54.
4. Hulme, E.C., et al. 1991. Muscarinic acetylcholine receptors: structure and function. *Biochem. Soc. Trans.* 19: 133-138.
5. Caulfield, M.P. 1993. Muscarinic receptor-characterization, coupling and function. *Pharmacol. Ther.* 58: 319-379.
6. Brann, M.R., et al. 1993. Muscarinic acetylcholine receptor subtypes: localization and structure/function. *Prog. Brain Res.* 98: 121-127.

## CHROMOSOMAL LOCATION

Genetic locus: CHRM3 (human) mapping to 1q43.

## PRODUCT

mAChR M3 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see mAChR M3 shRNA Plasmid (h): sc-35833-SH and mAChR M3 shRNA (h) Lentiviral Particles: sc-35833-V as alternate gene silencing products.

For independent verification of mAChR M3 (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-35833A, sc-35833B and sc-35833C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

mAChR M3 siRNA (h) is recommended for the inhibition of mAChR M3 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  $\mu$ M in 66  $\mu$ 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.

## GENE EXPRESSION MONITORING

mAChR M3 (H-4): sc-518107 is recommended as a control antibody for monitoring of mAChR M3 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>TM</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor mAChR M3 gene expression knockdown using RT-PCR Primer: mAChR M3 (h)-PR: sc-35833-PR (20  $\mu$ l, 505 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Schmitt, J.M., et al. 2010. ERK activation and cell growth require CaM kinases in MCF-7 breast cancer cells. *Mol. Cell. Biochem.* 335: 155-171.
2. Balaratnam, S., et al. 2018. A secondary structure within a human piRNA modulates its functionality. *Biochimie* 157: 72-80.

## RESEARCH USE

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

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.