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P2X7 siRNA (m): sc-42576

BACKGROUND

The P2X receptor family is comprised of ligand-gated ion channels that allow for the increased permeability of calcium into the cell in response to extracellular ATP. The seven P2X receptors, P2X1-P2X7, form either homomeric or heteromeric channels or both. They are characterized by intracellular amino- and carboxy-termini. P2X receptors are expressed in a wide variety of tissues, including neurons, prostate, bladder, pancreas, colon, testis and ovary. The major function of the P2X receptors is to mediate synaptic transmissions between neurons and to other tissues via the binding of extracellular ATP, which acts as a neurotransmitter. The P2X receptors may be involved in the onset of necrosis or apoptosis after prolonged exposure to high concentrations of extracellular ATP.

REFERENCES

1. Longhurst, P.A., et al. 1996. The human P2X1 receptor: molecular cloning, tissue distribution, and localization to chromosome 17. *Biochim. Biophys. Acta* 1308: 185-188.
2. Di Virgilio, F., et al. 1998. Cytolytic P2X purinoceptors. *Cell Death Differ.* 5: 191-199.
3. Alexander, K., et al. 1999. Allosteric modulation and accelerated resensitization of human P2X3 receptors by cibacron blue. *J. Pharmacol. Exp. Ther.* 291: 1135-1142.

CHROMOSOMAL LOCATION

Genetic locus: P2rx7 (mouse) mapping to 5 F.

PRODUCT

P2X7 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see P2X7 shRNA Plasmid (m): sc-42576-SH and P2X7 shRNA (m) Lentiviral Particles: sc-42576-V as alternate gene silencing products.

For independent verification of P2X7 (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-42576A, sc-42576B and sc-42576C.

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

P2X7 siRNA (m) is recommended for the inhibition of P2X7 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 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.

GENE EXPRESSION MONITORING

P2X7 (D-1): sc-514962 is recommended as a control antibody for monitoring of P2X7 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 P2X7 gene expression knockdown using RT-PCR Primer: P2X7 (m)-PR: sc-42576-PR (20 μ l, 491 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Zhao, J., et al. 2013. P2X7 blockade attenuates murine lupus nephritis by inhibiting activation of the NLRP3/ASC/caspase 1 pathway. *Arthritis Rheum.* 65: 3176-3185.
2. Yan, Y., et al. 2015. P2X7 receptor inhibition protects against ischemic acute kidney injury in mice. *Am. J. Physiol., Cell Physiol.* 308: C463-C472.
3. Sekar, P., et al. 2018. AMPK-dependent and independent actions of P2X7 in regulation of mitochondrial and lysosomal functions in microglia. *Cell Commun. Signal.* 16: 83.
4. Sekar, P., et al. 2018. Coordinate effects of P2X7 and extracellular acidification in microglial cells. *Oncotarget* 9: 12718-12731.
5. Yang, R., et al. 2018. Tet1 and Tet2 maintain mesenchymal stem cell homeostasis via demethylation of the P2rx7 promoter. *Nat. Commun.* 9: 2143.

RESEARCH USE

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