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P2Y1 siRNA (h): sc-42577

BACKGROUND

Nucleotides are emerging as important extracellular signaling molecules that mediate several effects, such as proliferation, differentiation, chemotaxis and cytokine release. The P2 receptor family is activated by the binding of nucleotides and is divided into two subfamilies, P2X and P2Y. 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 P2Y receptor family are G protein-coupled receptors which mediate the effects of extracellular nucleotides, primarily through the activation of phospholipase C. To some extent, the P2Y receptors can also activate potassium channels or, alternatively, inhibit adenylate cyclase and N-type calcium channels in response to extracellular nucleotides. The P2Y receptors are differentially expressed in several tissue in leukocytes, which suggests a role for the P2Y receptor family in the activation of leukocytes and platelets in response to inflammation or vascular damage.

REFERENCES

1. Akbar, G.K., et al. 1996. Molecular cloning of a novel P2 purinoceptor from human erythroleukemia cells. *J. Biol. Chem.* 271: 18363-18367.
2. North, R.A., et al. 1997. Nucleotide receptors. *Curr. Opin. Neurobiol.* 7: 346-357.
3. Burnstock, G. 2000. P2X receptors in sensory neurones. *Br. J. Anaesth.* 84: 476-488.
4. Oury, C., et al. 2000. A natural dominant negative P2X1 receptor due to deletion of a single amino acid residue. *J. Biol. Chem.* 275: 22611-22614.
5. Ding, S., et al. 2000. Inactivation of P2X2 purinoceptors by divalent cations. *J. Physiol.* 2: 199-214.
6. Adrian, K., et al. 2000. Expression of purinergic receptors (ionotropic P2X1-7 and metabotropic P2Y1-11) during myeloid differentiation of HL-60 cells. *Biochim. Biophys. Acta* 1492: 127-138.

CHROMOSOMAL LOCATION

Genetic locus: P2RY1 (human) mapping to 3q25.2.

PRODUCT

P2Y1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see P2Y1 shRNA Plasmid (h): sc-42577-SH and P2Y1 shRNA (h) Lentiviral Particles: sc-42577-V as alternate gene silencing products.

For independent verification of P2Y1 (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-42577A, sc-42577B and sc-42577C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

P2Y1 siRNA (h) is recommended for the inhibition of P2Y1 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 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

P2Y1 (E-1): sc-377324 is recommended as a control antibody for monitoring of P2Y1 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 P2Y1 gene expression knockdown using RT-PCR Primer: P2Y1 (h)-PR: sc-42577-PR (20 μ l, 526 bp). 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.