

P2Y12 siRNA (r): sc-108057

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. Human platelets express two G protein-coupled nucleotide receptors, P2Y1 and P2Y12. P2Y12 is a receptor for ADP and ATP coupled to G-proteins that inhibit the adenylate cyclase second messenger system. P2Y12 is an integral membrane protein involved in platelet aggregation. It is highly expressed in platelets, with lower levels in the brain, lung, appendix, pituitary and adrenal gland.

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. Di Virgilio, F., et al. 2001. Nucleotide receptors: an emerging family of regulatory molecules in blood cells. *Blood* 97: 587-600.
5. Simon, J., et al. 2002. Characterization and channel coupling of the P2Y(12) nucleotide receptor of brain capillary endothelial cells. *J. Biol. Chem.* 277: 31390-31400.
6. Ding, Z., et al. 2003. Inactivation of the human P2Y12 receptor by thiol reagents requires interaction with both extracellular cysteine residues, Cys17 and Cys270. *Blood* 101: 3908-3914.

CHROMOSOMAL LOCATION

Genetic locus: P2ry12 (rat) mapping to 2q26.

PRODUCT

P2Y12 siRNA (r) 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 P2Y12 shRNA Plasmid (r): sc-108057-SH and P2Y12 shRNA (r) Lentiviral Particles: sc-108057-V as alternate gene silencing products.

For independent verification of P2Y12 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-108057A, sc-108057B and sc-108057C.

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

P2Y12 siRNA (r) is recommended for the inhibition of P2Y12 expression in rat 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor P2Y12 gene expression knockdown using RT-PCR Primer: P2Y12 (r)-PR: sc-108057-PR (20 μ l). 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.