SANTA CRUZ BIOTECHNOLOGY, INC.

PAR-3 siRNA (m): sc-37144

BACKGROUND

Thrombin receptor (also designated protease-activated receptor-1 or PAR-1), PAR-2 and PAR-3 compose a distinct class of G protein-coupled receptors activated by proteolysis. Cleavage of these receptors by proteases occurs within the amino-terminal extracellular domain. Thrombin, a serine protease involved in platelet aggregation and blood coagulation, activates the thrombin receptor, resulting in elevated intracellular calcium levels in platelets. Thrombin also cleaves PAR-3 *in vitro,* suggesting that PAR-3 may be involved in thrombosis or mitogenesis. Thrombin receptor and PAR-4 appear to account for most thrombin signaling in platelets. Activation of PAR-2 *in vitro* is induced by trypsin, suggesting that PAR-2 is not an alternative thrombin receptor. Cytokines including TNF-α and IL-1β increase PAR-2 expression, indicating PAR-2 involvement in the acute inflammatory response.

REFERENCES

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2. Lerner, D.J., et al. 1996. Agonist recognition by proteinase-activated receptor-2 and Thrombin receptor. Importance of extracellular loop interactions for receptor function. J. Biol. Chem. 271: 13943-13947.
3. Nystedt, S., et al. 1996. The proteinase-activated receptor-2 is induced by inflammatory mediators in human endothelial cells. Comparison with the Thrombin receptor. J. Biol. Chem. 271: 14910-14915.
4. Xu, W.F., et al. 1998. Cloning and characterization of human protease-activated receptor-4. Proc. Natl. Acad. Sci. USA 95: 6642-6646.
5. Goldsack, N.R., et al. 1998. Thrombin. Int. J. Biochem. Cell Biol. 30: 641-646.
6. Sullivan, R., et al. 1998. Analysis of a Ca^{2+}-activated K^+ channel that mediates hyperpolarization via the Thrombin receptor pathway. Am. J. Physiol. 275: C1342-C1348.
7. Schmidt, V.A., et al. 1998. The human proteinase-activated receptor-3 (PAR-3) gene. Identification within a PAR gene cluster and characterization in vascular endothelial cells and platelets. J. Biol. Chem. 273: 15061-15068.
8. Kahn, M.L., et al. 1999. Protease-activated receptors-1 and -4 mediate activation of human platelets by Thrombin. J. Clin. Invest. 103: 879-887.

CHROMOSOMAL LOCATION

Genetic locus: F2rl2 (mouse) mapping to 13 D1.

PRODUCT

PAR-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 PAR-3 shRNA Plasmid (m): sc-37144-SH and PAR-3 shRNA (m) Lentiviral Particles: sc-37144-V as alternate gene silencing products.

For independent verification of PAR-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-37144A, sc-37144B and sc-37144C.

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

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

PAR-3 (G-4): sc-393127 is recommended as a control antibody for monitoring of PAR-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 PAR-3 gene expression knockdown using RT-PCR Primer: PAR-3 (m)-PR: sc-37144-PR (20 µl, 464 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.

PROTOCOLS

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