SANTA CRUZ BIOTECHNOLOGY, INC.

PAR-3 siRNA (h): sc-37143

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

1. Santulli, R.J., et al. 1995. Evidence for the presence of a protease-activated receptor distinct from the Thrombin receptor in human keratinocytes. Proc. Natl. Acad. Sci. USA 92: 9151-9155.
2. Lerner, D.J., et al. 1996. Agonist recognition by proteinase-activated receptor 2 and Thrombin receptor. Importance of extracellular loop inter-actions 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.

CHROMOSOMAL LOCATION

Genetic locus: F2rl2 (human) mapping to 5q13; F2rl2 (mouse) mapping to 13 D1.

PRODUCT

PAR-3 siRNA (h) is a pool of 3 target-specific 20-25 nt siRNAs designed to knock down gene expression. Each vial contains 3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections.

For independent verification of PAR-3 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3 nmol of lyophilized siRNA. These include: sc-37143A, sc-37143B and sc-37143C.

RESEARCH USE

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

PROTOCOLS

See our web site at www.scbt.com or our catalog 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 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 (h) is recommended for the inhibition of PAR-3 expression in human cells.

PAR-3 (H-103): sc-5598 is recommended as a control antibody for Western Blotting (starting dilution 1:100, dilution range 1:100-1:1,000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) protein detection using the recommended secondary reagents listed below.

RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 10µM in 60 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. Semi-quantitative RT-PCR may be performed using RT-PCR Primer: PAR-3 (h)-PR: sc-37143-PR (20 µ, 458 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.