

PAR-4 siRNA (m): sc-72069

BACKGROUND

Thrombin receptor (also designated protease-activated receptor-1 or PAR-1), PAR-2, PAR-3 and PAR-4 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-1 β 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 interactions for receptor function. *J. Biol. Chem.* 271: 13943-13947.
3. Xu, W.F., et al. 1998. Cloning and characterization of human protease-activated receptor 4. *Proc. Natl. Acad. Sci. USA* 95: 6642-6646.
4. Goldsack, N.R., et al. 1998. Thrombin. *Int. J. Biochem. Cell Biol.* 30: 641-646.

CHROMOSOMAL LOCATION

Genetic locus: F2rl3 (mouse) mapping to 8 B3.3.

PRODUCT

PAR-4 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 PAR-4 shRNA Plasmid (m): sc-72069-SH and PAR-4 shRNA (m) Lentiviral Particles: sc-72069-V as alternate gene silencing products.

For independent verification of PAR-4 (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-72069A, sc-72069B and sc-72069C.

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

PAR-4 siRNA (m) is recommended for the inhibition of PAR-4 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

PAR4 (A-10): sc-1666 is recommended as a control antibody for monitoring of PAR-4 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 MarkerTM 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 PAR-4 gene expression knockdown using RT-PCR Primer: PAR-4 (m)-PR: sc-72069-PR (20 μ l, 422 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Azmi, A.S., et al. 2008. Critical role of prostate apoptosis response-4 in determining the sensitivity of pancreatic cancer cells to small-molecule inhibitor-induced apoptosis. *Mol. Cancer Ther.* 7: 2884-2893.
2. Han, J.Y., et al. 2016. The role of prostate apoptosis response-4 (Par-4) in *Mycobacterium tuberculosis* infected macrophages. *Sci. Rep.* 6: 32079.

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.