SANTA CRUZ BIOTECHNOLOGY, INC.

Thrombin R siRNA (h): sc-36663

BACKGROUND

Thrombin is a serine protease that is involved in platelet aggregation and blood coagulation. It is cleaved from its precursor, Prothrombin, and converts Fibrinogen to Fibrin in the final step of the clotting cascade. Thrombin mediates its regulatory effects by activating cell surface receptors. These receptors, including Thrombin R (also designated PAR-1, for protease-activated receptor-1), PAR-2 and PAR-3, are members of the G protein-coupled receptor family, and share a similar gene structure. Thrombin cleaves its receptor, releasing a 41 amino acid peptide that acts as a platelet agonist. Upon this activation by thrombin, the Thrombin Rs trigger an increase in cytosolic Ca^{2+} concentration. Unactivated Thrombin R cycles between the cell surface and an intracellular pool, while activated Thrombin R internalizes rapidly and is degraded in the lysosomes. The human Thrombin R is also known to be regulated by Sp1 and Sp3 transcription factors.

REFERENCES

1. Goldsack, N.R., et al. 1998. Thrombin. Int. J. Biochem. Cell Biol. 30: 641-646.
2. Kahn, M.L., et al. 1998. Gene and locus structure and chromosomal localization of the protease-activated receptor gene family. J. Biol. Chem. 273: 23290-23296.
3. Furman, M.I., et al. 1998. The cleaved peptide of the thrombin receptor is a strong platelet agonist. Proc. Natl. Acad. Sci. USA 95: 3082-3087.

CHROMOSOMAL LOCATION

Genetic locus: F2R (human) mapping to 5q13.3.

PRODUCT

Thrombin R 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Thrombin R shRNA Plasmid (h): sc-36663-SH and Thrombin R shRNA (h) Lentiviral Particles: sc-36663-V as alternate gene silencing products.

For independent verification of Thrombin R (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-36663A, sc-36663B and sc-36663C.

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

Thrombin R siRNA (h) is recommended for the inhibition of Thrombin R 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 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

Thrombin R (ATAP2): sc-13503 is recommended as a control antibody for monitoring of Thrombin R 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Thrombin R gene expression knockdown using RT-PCR Primer: Thrombin R (h)-PR: sc-36663-PR (20 µl, 414 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Liu, J., et al. 2004. Double transfection improves small interfering RNA-induced thrombin receptor (PAR-1) gene silencing in DU 145 prostate cancer cells. FEBS Lett. 577: 175-180.
2. Liu, J.F., et al. 2012. Thrombin induces Heme Oxygenase-1 expression in human synovial fibroblasts through protease-activated receptor signaling pathways. Arthritis Res. Ther. 14: R91.
3. Mazor, R., et al. 2013. Matrix metalloproteinase-1-mediated up-regulation of vascular endothelial growth factor-2 in endothelial cells. J. Biol. Chem. 288: 598-607.
4. d'Audigier, C., et al. 2015. Thrombin receptor PAR-1 activation on endothelial progenitor cells enhances chemotaxis-associated genes expression and leukocyte recruitment by a Cox-2-dependent mechanism. Angiogenesis 18: 347-359.
5. Nieuwenhuizen, L., et al. 2016. Silencing of protease-activated receptors attenuates synovitis and cartilage damage following a joint bleed in haemophilic mice. Haemophilia 22: 152-159.
6. Kim, S., et al. 2018. PAR-1 is a novel mechano-sensor transducing laminar flow-mediated endothelial signaling. Sci. Rep. 8: 15172.
7. Natarajan, K., et al. 2019. Organic dust induces inflammatory gene expression in lung epithelial cells via ROS-dependent Stat3 activation. Am. J. Physiol. Lung Cell. Mol. Physiol. 317: L127-L140.

RESEARCH USE

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