SANTA CRUZ BIOTECHNOLOGY, INC.

PAR-4 siRNA (h): sc-72068

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-1β increase PAR-2 expression, indicating PAR-2 involvement in the acute inflammatory response.

CHROMOSOMAL LOCATION

Genetic locus: F2RL3 (human) mapping to 19p13.11.

PRODUCT

PAR-4 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 PAR-4 shRNA Plasmid (h): sc-72068-SH and PAR-4 shRNA (h) Lentiviral Particles: sc-72068-V as alternate gene silencing products.

For independent verification of PAR-4 (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-72068A, sc-72068B and sc-72068C.

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

PAR-4 (5F4): sc-293206 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-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-4 gene expression knockdown using RT-PCR Primer: PAR-4 (h)-PR: sc-72068-PR (20 µl, 416 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. Mambetsariev, N., et al. 2010. Hyaluronic Acid binding protein 2 is a novel regulator of vascular integrity. Arterioscler. Thromb. Vasc. Biol. 30: 483-490.
3. Chen, H.T., et al. 2010. Thrombin enhanced migration and MMPs expression of human chondrosarcoma cells involves PAR receptor signaling pathway. J. Cell. Physiol. 223: 737-745.
4. Dangwal, S., et al. 2011. High glucose enhances thrombin responses via protease-activated receptor-4 in human vascular smooth muscle cells. Arterioscler. Thromb. Vasc. Biol. 31: 624-633.
5. Huang, C.Y., et al. 2012. Thrombin induces epidermal growth factor receptor transactivation and CCL2 expression in human osteoblasts. Arthritis Rheum. 64: 3344-3354.
6. Chaudhry, P., et al. 2014. Prostate apoptosis response-4 mediates TGF-β-induced epithelial-to-mesenchymal transition. Cell Death Dis. 5: e1044.
7. 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.
8. Das, T.P., et al. 2016. Inhibition of AKT promotes FOXO3a-dependent apoptosis in prostate cancer. Cell Death Dis. 7: e2111.

RESEARCH USE

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