SANTA CRUZ BIOTECHNOLOGY, INC.

PAR4 siRNA (m): sc-36189

BACKGROUND

Normal tissues are characterized by a balance between cellular stasis, cell proliferation, cell differentiation and cell death. Aberrant regulation of any of these cell processes can result in cancer. Cell death during embryogenesis, tissue atrophy and normal tissue turnover is called apoptosis and is characterized by cytoplasmic and nuclear condensation, nuclear disorganization and fragmentation of genomic DNA into 180-200 base pair oligomers. Five ionomycin-inducible complementary cDNAs, designated PAR1, 2, 3, 4 and 5, have been isolated from the prostate cancer cell line AT-3. Nucleotide sequencing identified PAR1 as the rat homolog of MKP-1, PAR2 as the injury-inducible gene HB-EGF, and PAR3 as the serum-induced gene Cyr61. PAR4 and PAR5 sequences were not found to correspond to any previously described proteins. PAR4 (prostate apoptosis response 4) is specifically expressed by cells entering apoptosis and is not induced during growth factor stimulation, oxidative stress, necrosis or growth arrest. The PAR4 gene encodes a protein with a putative nuclear localization signal and carboxy terminal leucine zipper.

REFERENCES

1. Herrmann, J.L., et al. 1998. Prostate carcinoma cell death resulting from inhibition of proteasome activity is independent of functional Bcl-2 and p53. Oncogene 17: 2889-2899.
2. Fioretti, B., et al. 2004. Histamine activates a background, arachidonic acid-sensitive K channel in embryonic chick dorsal root ganglion neurons. Neuroscience 125: 119-127.
3. Wang, G., et al. 2005. Direct binding to ceramide activates protein kinase Cζ before the formation of a pro-apoptotic complex with PAR4 in differentiating stem cells. J. Biol. Chem. 280: 26415-26424.
4. Park, S.K., et al. 2005. PAR4 links dopamine signaling and depression. Cell 122: 275-287.
5. Luke, M.P., et al. 2006. Targeted ablation of PAR4 reveals a cell type-specific susceptibility to apoptosis-inducing agents. Cancer Res. 66: 3456-3462.
6. Welters, H., et al. 2006. Conditional expression of hepatocyte nuclear factor-1β, the maturity-onset diabetes of the young-5 gene product, influences the viability and functional competence of pancreatic β-cells. J. Endocrinol. 190: 171-181.

CHROMOSOMAL LOCATION

Genetic locus: Pawr (mouse) mapping to 10 D1.

PRODUCT

PAR4 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 PAR4 shRNA Plasmid (m): sc-36189-SH and PAR4 shRNA (m) Lentiviral Particles: sc-36189-V as alternate gene silencing products.

For independent verification of PAR4 (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-36189A, sc-36189B and sc-36189C.

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

PAR4 siRNA (m) is recommended for the inhibition of PAR4 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

PAR4 (A-10): sc-1666 is recommended as a control antibody for monitoring of PAR4 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 PAR4 gene expression knockdown using RT-PCR Primer: PAR4 (m)-PR: sc-36189-PR (20 µl, 476 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.