SANTA CRUZ BIOTECHNOLOGY, INC.

PI-9 siRNA (h): sc-40949

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

Serine proteinase inhibitors (serpins) function as regulators of serine proteinase activity in a variety of physiological processes. Proteinase inhibitor-9 (PI-9, also designated cytoplasmic antiproteinase 3, or CAP3) is a member of the Ovalbumin family of serpins that is expressed in placenta, lung and cytotoxic lymphocytes. PI-9 is a potent inhibitor of granzyme B and of granzyme B-mediated apoptosis, and is also an inhibitor of caspase-1 and, to a lesser extent, caspase-4 and caspase-8. Because granzyme B promotes DNA degradation and rapidly translocates to the nucleus to bind to a nuclear component, PI-9 is present in the nuclei of human cytotoxic cells, endothelial cells and epithelial cells. PI-9 is exported from nuclei via a leptomycin B-sensitive pathway, suggesting that the nucleocytoplasmic distribution of PI-9 involves a non-conventional nuclear import pathway and the export factor CRM1. Estrogen rapidly and strongly induces PI-9, which is an estrogen-regulated human gene. PI-9 expression is also upregulated in response to inflammatory stimuli. This upregulation protects cells from apoptosis induced by endogenously expressed or released granzyme B, particulary during target cell killling. In addition, PI-9 is expressed in a variety of human and murine tumors.

REFERENCES

1. Dahlen, J.R., et al. 1997. Human proteinase inhibitor-9 (PI-9) is a potent inhibitor of subtilisin A. Biochem. Biophys. Res. Commun. 238: 329-333.
2. Sun, J., et al. 1997. A new family of 10 murine Ovalbumin serpins includes two homologs of proteinase inhibitor-8 and two homologs of the granzyme B inhibitor (proteinase inhibitor-9). J. Biol. Chem. 272: 15434-15441.
3. Sun, J., et al. 1998. A serpin gene cluster on human chromosone 6p25 contains PI-6, PI-9 and ELANH$_2$ which have a common structure almost identical to the 18q21 Ovalbumin serpin genes. Cytogenet. Cell Genet. 82: 273-277.
4. Dahlen, J.R., et al. 1999. Inhibition of neutrophil elastase by recombinant human proteinase inhibitor-9. Biochem. Biophys. Acta 1451: 233-241.
5. Annand, R.R., et al. 1999. Caspase-1 (Interleukin-1β-converting enzyme) is inhibited by the human serpin analogue proteinase inhibitor-9. Biochem. J. 342: 655-665.
6. Kanamori, H., et al. 2000. Proteinase inhibitor 9, an inhibitor of granzyme B-mediated apoptosis, is a primary estrogen-inducible gene in human liver cells. J. Biol. Chem. 275: 5867-5873.

CHROMOSOMAL LOCATION

Genetic locus: SERPINB9 (human) mapping to 6p25.2.

PRODUCT

PI-9 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 PI-9 shRNA Plasmid (h): sc-40949-SH and PI-9 shRNA (h) Lentiviral Particles: sc-40949-V as alternate gene silencing products.

For independent verification of PI-9 (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-40949A, sc-40949B and sc-40949C.

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

PI-9 siRNA (h) is recommended for the inhibition of PI-9 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

PI-9 (C-10): sc-390406 is recommended as a control antibody for monitoring of PI-9 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 PI-9 gene expression knockdown using RT-PCR Primer: PI-9 (h)-PR: sc-40949-PR (20 µl). 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.