SANTA CRUZ BIOTECHNOLOGY, INC.

granzyme B siRNA (h): sc-35507

BACKGROUND

Granzyme A and granzyme B are serine proteases that mediate apoptotic signaling in cytotoxic T lymphocytes (CTL) and natural killer (NK) cells. Both granzyme A and granzyme B are synthesized as inactive proenzymes, and they are stored within cytolytic granules and released by effector cells during degranulation. In activated CTLs, granzyme A and granzyme B are processed and activated by cathepsin C, and they then function to induce apoptosis by two distinct pathways. Granzyme B proteolytically cleaves and activates members of the caspase family of cysteine proteases, including caspase-3, caspase-6, caspase-7 and caspase-9. When cleaved, these caspases assemble into active holoenzymes that then mediate apoptosis through a defined proteolytic cascade involving nuclear lamins and PARP (poly ADP ribose polymerase). Granzyme A mediates the activation of apoptosis by inducing single-strand DNA breaks, membrane perturbation and nuclear condensations in an alternative pathway that is independent from caspase activation or the caspase proteolytic cascade.

REFERENCES

1. Gershenfeld, H.K., et al. 1988. Cloning and chromosomal assignment of a human cDNA encoding a T cell- and natural killer cell-specific trypsin-like serine protease. Proc. Natl. Acad. Sci. USA 85: 1184-1188.
2. Shresta, S., et al. 1995. Natural killer and lymphokine-activated killer cells require granzyme B for the rapid induction of apoptosis in susceptible target cells. Proc. Natl. Acad. Sci. USA 92: 5679-5683.
3. Trapani, J.A., et al. 1996. A putative role in the mechanism of cytotoxic lymphocyte-mediated apoptosis. Localization of granzyme B in the nucleus. J. Biol. Chem. 271: 4127-4133.
4. Atkinson, E.A., et al. 1998. Cytotoxic T lymphocyte-assisted suicide. Caspase 3 activation is primarily the result of the direct action of granzyme B. J. Biol. Chem. 273: 21261-21266.
5. Trapani, J.A., et al. 1998. Efficient nuclear targeting of granzyme B and the nuclear consequences of apoptosis induced by granzyme B and perforin are caspase-dependent, but cell death is caspase-independent. J. Biol. Chem. 273: 27934-27938.

CHROMOSOMAL LOCATION

Genetic locus: GZMB (human) mapping to 14q12.

PRODUCT

granzyme B 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 granzyme B shRNA Plasmid (h): sc-35507-SH and granzyme B shRNA (h) Lentiviral Particles: sc-35507-V as alternate gene silencing products.

For independent verification of granzyme B (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-35507A, sc-35507B and sc-35507C.

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

granzyme B siRNA (h) is recommended for the inhibition of granzyme B 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

granzyme B (2C5): sc-8022 is recommended as a control antibody for monitoring of granzyme B 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 granzyme B gene expression knockdown using RT-PCR Primer: granzyme B (h)-PR: sc-35507-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.