SANTA CRUZ BIOTECHNOLOGY, INC.

granzyme M siRNA (m): sc-60762

BACKGROUND

The granzyme family of proteins belong to the larger peptidase S_1 family. Granzyme A and granzyme B are serine proteases that facilitate apoptotic signaling in cytotoxic T lymphocytes (CTL) and natural killer (NK) cells. Within the granules of activated CTLs, granzyme A and granzyme B are processed and converted to their active forms by the lysosomal cysteine protease cathepsin C. Once cleaved, these active proteases target distinct substrates for proteolysis, thereby mediating apoptosis through two different pathways. Granzyme H localizes to cytoplasmic granules of cytolytic T lymphocytes and is important for target cell lysis in cell-mediated immune responses. Granzyme K is a serine protease localizing to the granules of NK cells and cytotoxic T lymphocytes. Granzyme M (also designated NK cell granular protease, HU-Met-1 or met-1 serine protease) contains one peptidase S_1 domain. Granzyme M is a Trypsin-fold serine protease that localizes to granules of large granular lymphocytes (NK cells) and cleaves peptide substrates after leucine, norleucine or methionine. This enzyme may play a role in target cell death induction by cytotoxic lymphocytes.

REFERENCES

1. Mahrus, S., et al. 2004. Granzyme M is a regulatory protease that inactivates proteinase inhibitor 9, an endogenous inhibitor of granzyme B. J. Biol. Chem. 279: 54275-54282.
2. Bade, B., et al. 2005. Differential expression of the granzymes A, K and M and perforin in human blood lymphocytes. Int. Immunol. 17: 1419-1428.
3. Pao, L.I., et al. 2005. Functional analysis of granzyme M and its role in immunity to infection. J. Immunol. 175: 3235-3243.
4. Suck, G., et al. 2005. KHYG-1, a model for the study of enhanced natural killer cell cytotoxicity. Exp. Hematol. 33: 1160-1171.
5. Bots, M., et al. 2005. SPI-CI and SPI-6 cooperate in the protection from effector cell-mediated cytotoxicity. Blood 105: 1153-1161.
6. Bots, M., et al. 2006. Serpins prevent granzyme-induced death in a species-specific manner. Immunol. Cell Biol. 84: 79-86.

CHROMOSOMAL LOCATION

Genetic locus: Gzmm (mouse) mapping to 10 C1.

PRODUCT

granzyme M 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 granzyme M shRNA Plasmid (m): sc-60762-SH and granzyme M shRNA (m) Lentiviral Particles: sc-60762-V as alternate gene silencing products.

For independent verification of granzyme M (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-60762A, sc-60762B and sc-60762C.

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 M siRNA (m) is recommended for the inhibition of granzyme M 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

granzyme M (A-10): sc-376799 is recommended as a control antibody for monitoring of granzyme M 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 M gene expression knockdown using RT-PCR Primer: granzyme M (m)-PR: sc-60762-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.