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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

SANTA CRUZ BIOTECHNOLOGY, INC.

Perforin 1 siRNA (m): sc-42593

BACKGROUND

The major defense of the body against virus-infected and tumorigenic cells is cytotoxic T lymphocyte (CTL)-mediated cytotoxicity, which also plays a role in autoimmune diseases and transplant rejection. During CTL-mediated cytotoxicity, CTL granules containing perforin are exocytosed. Perforin is a pore-forming protein that facilitates the entry of cytotoxic serine proteases, such as granzymes, into target cells by forming transmembrane channels in target cell membranes. Perforin is primarily expressed in cytotoxic T lymphocytes (CTL) and natural killer (NK) cells, but has also been observed in an astrocyte population of the human brain. It has been shown that abrogation of perforin function by Ca^{2+}-complexing agents leads to decreased levels of necrosis, demonstrating that both necrosis and apoptosis contribute to CTL-mediated cytotoxicity. Perforin activity has been shown to be induced by IL-2, IL-3, IL-4, IL-6 and to a lesser degree, TNF and IFN-γ.

REFERENCES

1. Liu, C.C., et al. 1990. Induction of perforin and serine esterases in a murine cytotoxic T lymphocyte clone. J. Immunol. 144: 1196-1201.
2. Podack, E.R., et al. 1991. A central role of perforin in cytolysis? Annu. Rev. Immunol. 9: 129-157.
3. Thia, K.Y., et al. 1993. Expression of human perforin in a mouse cytotoxic T lymphocyte cell line: evidence for perturbation of granule-mediated cytotoxicity. J. Leukoc. Biol. 54: 528-533.
4. Trapani, J.A. 1995. Target cell apoptosis induced by cytotoxic T cells and natural killer cells involves synergy between the pore-forming protein, perforin, the serine protease, granzyme B. Aust. N. Z. J. Med. 25: 793-799.
5. Darmon, A.J., et al. 1995. Activation of the apoptotic protease CPP32 by cytotoxic T-cell-derived granzyme B. Nature 377: 446-448.
6. Renner, C., et al. 1997. Role of perforin, granzymes and the proliferative state of the target cells in apoptosis and necrosis mediated by bispecific-antibody-activated cytotoxic T cells. Cancer Immunol. Immunother. 44: 70-76.
7. Gasque, P., et al. 1998. Identification of an astrocyte cell population from human brain that expresses perforin, a cytotoxic protein implicated in immune defense. J. Exp. Med. 187: 451-460.

CHROMOSOMAL LOCATION

Genetic locus: Prf1 (mouse) mapping to 10 B4.

PRODUCT

Perforin 1 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 Perforin 1 shRNA Plasmid (m): sc-42593-SH and Perforin 1 shRNA (m) Lentiviral Particles: sc-42593-V as alternate gene silencing products.

For independent verification of Perforin 1 (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-42593A, sc-42593B and sc-42593C.

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

Perforin 1 siRNA (m) is recommended for the inhibition of Perforin 1 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

Perforin 1 (F-1): sc-136994 is recommended as a control antibody for monitoring of Perforin 1 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 Perforin 1 gene expression knockdown using RT-PCR Primer: Perforin 1 (m)-PR: sc-42593-PR (20 µl, 501 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Weerasinghe, P., et al. 2013. A model for cardiomyocyte cell death: insights into mechanisms of oncosis. Exp. Mol. Pathol. 94: 289-300.

RESEARCH USE

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