SANTA CRUZ BIOTECHNOLOGY, INC.

SPAM1 siRNA (h): sc-60822

BACKGROUND

Hyaluronidases (HAases or HYALs) are a family of lysosomal enzymes that are crucial for the spread of bacterial infections and of toxins present in a variety of venoms. HYALs may also be involved in the progression of cancer. In humans, six members of the hyaluronidase family have been identified. These proteins are significant in the degradation of hyaluronic acid (HA), which is present in body fluids, tissues and the extracellular matrix of vertebrate tissues. HA keeps tissues hydrated, maintains osmotic balance and promotes cell proliferation, differentiation and metastasis. HA is also an important structural component of cartilage and other tissues and acts as a lubricant in joints. SPAM1 (sperm adhesion molecule 1), also designated Hyal-PH20 or sperm surface protein PH-20, was formerly referred to as HYAL1 (HYA1). The current nomenclature references the functional hyaluronidase activity of SPAM1 to permit acrosome-intact sperm to penetrate through the HA-rich cumulus cell layer surrounding the oocyte. SPAM1 is also implicated in intracellular signaling and zona pellucida binding. SPAM1 is found in the epididymis and in testis, more specifically in plasma and acrosomal membranes of sperm. SPAM1 is a structurally unique hyaluronidase in that it is a GPI-anchored protein.

REFERENCES

1. Lathrop, W.F., et al. 1990. cDNA cloning reveals the molecular structure of a sperm surface protein, PH-20, involved in sperm-egg adhesion and the wide distribution of its gene among mammals. J. Cell Biol. 111: 2939-2949.
2. Lin, Y., et al. 1993. Molecular cloning of the human and monkey sperm surface protein PH-20. Proc. Natl. Acad. Sci. USA 90: 10071-10075.
3. Gmachl, M., et al. 1993. The human sperm protein PH-20 has hyaluronidase activity. FEBS Lett. 336: 545-548.
4. Lin, Y., et al. 1994. A hyaluronidase activity of the sperm plasma membrane protein PH-20 enables sperm to penetrate the cumulus cell layer surrounding the egg. J. Cell Biol. 125: 1157-1163.
5. Jones, M.H., et al. 1995. Expression analysis, genomic structure, and mapping to 7q31 of the human sperm adhesion molecule gene SPAM1. Genomics 29: 796-800.
6. Isoyama, T., et al. 2005. Differential selectivity of hyaluronidase inhibitors toward acidic and basic hyaluronidases. Glycobiology 16: 11-21.

CHROMOSOMAL LOCATION

Genetic locus: SPAM1 (human) mapping to 7q31.32.

PRODUCT

SPAM1 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 SPAM1 shRNA Plasmid (h): sc-60822-SH and SPAM1 shRNA (h) Lentiviral Particles: sc-60822-V as alternate gene silencing products.

For independent verification of SPAM1 (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-60822A, sc-60822B and sc-60822C.

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

SPAM1 siRNA (h) is recommended for the inhibition of SPAM1 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

SPAM1 (D-9): sc-518256 is recommended as a control antibody for monitoring of SPAM1 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 SPAM1 gene expression knockdown using RT-PCR Primer: SPAM1 (h)-PR: sc-60822-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.