SANTA CRUZ BIOTECHNOLOGY, INC.

ASTL siRNA (h): sc-94887

BACKGROUND

ASTL (astacin-like metalloendopeptidase), also known as ovastacin, is a 435 amino acid protein that belongs to the astacin family of metalloproteases. The human ASTL, which shares a 78% sequence identity with mouse ASTL, contains an N-terminal signal peptide, a prodomain, a zinc-dependent metalloprotease domain and a C-terminal extension that is likely to be heavily O-glycosylated. Highly expressed in unfertilized oocytes, ASTL expression drops to undetectable levels upon fertilization. ASTL has also been shown to be under hormonal regulation, as superovulation caused a dramatic increase in the expression of ASTL. The catalytic activity of ASTL is inhibited by EDTA and the wide spectrum metalloproteinase inhibitor batimastat (BB-94). The gene encoding ASTL maps to chromosome 2q11.1. Two isoforms of ASTL2 exist as a result of alternative splicing events.

REFERENCES

1. Stöcker, W., et al. 1993. Implications of the three-dimensional structure of astacin for the structure and function of the astacin family of zinc-endopeptidases. Eur. J. Biochem. 214: 215-231.
2. Bond, J.S., et al. 1995. The astacin family of metalloendopeptidases. Protein Sci. 4: 1247-1261.
3. Quesada, V., et al. 2004. Identification and characterization of human and mouse ovastacin: a novel metalloproteinase similar to hatching enzymes from arthropods, birds, amphibians, and fish. J. Biol. Chem. 279: 26627-26634.
4. Online Mendelian Inheritance in Man, OMIM™. 2004. Johns Hopkins University, Baltimore, MD. MIM Number: 608860. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. Semenova, S.A., et al. 2008. The astacin family of metalloproteinases. Biomed. Khim. 54: 531-554.
6. Sterchi, E.E., 2008. Special issue: metzincin metalloproteinases. Mol. Aspects Med. 29: 255-257.

CHROMOSOMAL LOCATION

Genetic locus: ASTL (human) mapping to 2q11.1.

PRODUCT

ASTL siRNA (h) is a pool of 2 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 ASTL shRNA Plasmid (h): sc-94887-SH and ASTL shRNA (h) Lentiviral Particles: sc-94887-V as alternate gene silencing products.

For independent verification of ASTL (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-94887A and sc-94887B.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

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

ASTL (D-8): sc-514054 is recommended as a control antibody for monitoring of ASTL 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 ASTL gene expression knockdown using RT-PCR Primer: ASTL (h)-PR: sc-94887-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.