

Hepsin siRNA (h): sc-60782

BACKGROUND

Extracellular proteases mediate the digestion of neighboring extracellular matrix components in initial tumor growth, allow desquamation of tumor cells into the surrounding environment, provide the basis for invasion of basement membranes in targeted metastatic organs and are required for release and activation of many growth and angiogenic factors. Hepsin (also known as TMPRSS1) is a type II transmembrane serine protease in mammalian cells that is highly expressed on the surface of hepatocytes. Hepsin is frequently overexpressed in several tumors, suggesting that it is a candidate protease in the invasive process and growth capacity of tumor cells. The basal promoter region of the Hepsin gene contains potential binding sites for SP1, AP2, C/EBP, LF-A1 and E box, which may be responsible for the ubiquitous expression of the protein, which shows preferential expression in liver and kidney. Hepsin is located at the plasma membrane, with its catalytic subunit (C-terminal half) at the cell surface and its N-terminus facing the cytosol. Hepsin has been shown to play a role in normal cell growth, embryo-genesis, hepatocyte growth, blood coagulation and fertilization. In addition, Hepsin converts zymogen Factor VII to Factor VIIa, which is capable of initiating a coagulation pathway on the cell surface and ultimately leads to Thrombin formation.

REFERENCES

1. Tsuji, A., et al. 1991. Characterization of Hepsin, a membrane bound protease. *Biomed. Biochim. Acta* 50: 791-793.
2. Tsuji, A., et al. 1991. Hepsin, a cell membrane-associated protease. Characterization, tissue distribution, and gene localization. *J. Biol. Chem.* 266: 16948-16953.
3. Tanimoto, H., et al. 1997. Hepsin, a cell surface serine protease identified in hepatoma cells, is overexpressed in ovarian cancer. *Cancer Res.* 57: 2884-2887.

CHROMOSOMAL LOCATION

Genetic locus: HPN (human) mapping to 19q13.12.

PRODUCT

Hepsin 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Hepsin shRNA Plasmid (h): sc-60782-SH and Hepsin shRNA (h) Lentiviral Particles: sc-60782-V as alternate gene silencing products.

For independent verification of Hepsin (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-60782A, sc-60782B and sc-60782C.

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.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Hepsin siRNA (h) is recommended for the inhibition of Hepsin 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 μ M in 66 μ l. 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

Hepsin (2D5): sc-517056 is recommended as a control antibody for monitoring of Hepsin 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 Hepsin gene expression knockdown using RT-PCR Primer: Hepsin (h)-PR: sc-60782-PR (20 μ l, 474 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.