SANTA CRUZ BIOTECHNOLOGY, INC.

HGFA siRNA (m): sc-39569

BACKGROUND

Hepatocyte growth factor (HGF) is a pleiotropic growth factor variously designated scatter factor, hematopoietin A and mammary growth factor. HGF is synthesized as a single chain, 728 amino acid precursor with a 29 amino acid signal peptide which is not present in the mature protein. Biologically active HGF is composed of a disulfide-linked α chain and a β chain, both of which are highly glycosylated. HGF exerts its biological effects through the HGF receptor, c-Met, which is expressed in ovary and endometrial endothelium and in the basal layers of skin. Hepatocyte growth factor activator (HGFA) is a serine protease which functions to cleave single chain HGF to its active heterodimeric form. HGFA is specific to the liver. HGFA of human origin is synthesized as an inactive secreted 655 amino acid precursor which is activated to generate a heterodimer consisting of a 35 amino acid short chain and a 248 amino acid long chain linked together by a disulfide bond. The gene encoding HGFA maps to human chromosome 4p16.3.

REFERENCES

1. Miyazawa, K., et al. 1993. Molecular cloning and sequence analysis of the cDNA for a human serine protease responsible for activation of hepatocyte growth factor. Structural similarity of the protease precursor to blood coagulation Factor XII. J. Biol. Chem. 268: 10024-10028.
2. Shimomura, T., et al. 1993. Activation of the zymogen of hepatocyte growth factor activator by Thrombin. J. Biol. Chem. 268: 22927-22932.
3. Miyazawa, K., et al. 1994. Proteolytic activation of hepatocyte growth factor in response to tissue injury. J. Biol. Chem. 269: 8966-8970.
4. Naldini, L., et al. 1995. Biological activation of pro-HGF (hepatocyte growth factor) by urokinase is controlled by a stoichiometric reaction. J. Biol. Chem. 270: 603-611.
5. Niranjan, B., et al. 1995. HGF/SF: a potent cytokine for mammary growth, morphogenesis and development. Development 121: 2897-2908.
6. Ferracini, R., et al. 1995. The Met/HGF receptor is over-expressed in human osteosarcomas and is activated by either a paracrine or an autocrine circuit. Oncogene 10: 739-749.
7. Tuck, A.B., et al. 1996. Coexpression of hepatocyte growth factor and receptor (Met) in human breast carcinoma. Am. J. Pathol. 148: 225-232.

CHROMOSOMAL LOCATION

Genetic locus: Hgfac (mouse) mapping to 5 B2.

PRODUCT

HGFA 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 HGFA shRNA Plasmid (m): sc-39569-SH and HGFA shRNA (m) Lentiviral Particles: sc-39569-V as alternate gene silencing products.

For independent verification of HGFA (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-39569A, sc-39569B and sc-39569C.

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

HGFA siRNA (m) is recommended for the inhibition of HGFA 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

HGFA (B-6): sc-515126 is recommended as a control antibody for monitoring of HGFA 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 HGFA gene expression knockdown using RT-PCR Primer: HGFA (m)-PR: sc-39569-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.