

HGF α / β siRNA (r): sc-77324

BACKGROUND

Hepatocyte growth factor, or HGF, is a pleiotropic growth factor variously designated as 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 by normal hepatocytes, gastric and intestinal epithelium, ovarian and endometrial endothelium and in the basal layers of skin. While c-Met is not thought to be expressed in normal lung, thyroid or pancreatic tissue, c-Met has been detected in tumors originating from such tissue. The c-Met proto-oncogene encodes a 1408 amino acid glycoprotein that represents the prototypic member of a novel family of receptor tyrosine kinases (RTKs) that include Ron, Sea and Sex.

REFERENCES

1. Miyazawa, K., et al. 1994. Proteolytic activation of hepatocyte growth factor in response to tissue injury. *J. Biol. Chem.* 269: 8966-8970.
2. Niranjana, B., et al. 1995. HGF/SF: a potent cytokine for mammary growth, morphogenesis and development. *Development* 121: 2897-2908.
3. 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.
4. 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.
5. Tuck, A.B., et al. 1996. Coexpression of hepatocyte growth factor and receptor (Met) in human breast carcinoma. *Am. J. Pathol.* 148: 225-232.
6. Huff, J.L., et al. 1996. Expression and maturation of the cellular sea receptor, a member of the hepatocyte growth factor (HGF) receptor family of protein tyrosine kinases. *Oncogene* 12: 299-307.
7. Maestrini, E., et al. 1996. A family of transmembrane proteins with homology to the Met-hepatocyte growth factor receptor. *Proc. Natl. Acad. Sci. USA* 93: 674-678.

CHROMOSOMAL LOCATION

Genetic locus: Hgf (rat) mapping to 4q12.

PRODUCT

HGF α / β siRNA (r) 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 HGF α / β shRNA Plasmid (r): sc-77324-SH and HGF α / β shRNA (r) Lentiviral Particles: sc-77324-V as alternate gene silencing products.

For independent verification of HGF α / β (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-77324A, sc-77324B and sc-77324C.

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

HGF α / β siRNA (r) is recommended for the inhibition of HGF α / β expression in rat 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

HGF α (H-10): sc-374422 is recommended as a control antibody for monitoring of HGF α / β 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 HGF α / β gene expression knockdown using RT-PCR Primer: HGF α / β (r)-PR: sc-77324-PR (20 μ l). 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.