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ITI-H5 siRNA (m): sc-146309

BACKGROUND

ITI-H5 (inter- α (globulin) inhibitor H5), also known as inter- α -trypsin inhibitor heavy chain H5, is a 942 amino acid protein belonging to the ITIH family. Encoded by a gene that maps to human chromosome 10p14, ITI-H5 is differentially expressed in human breast tumor and ovary tissues, with highest levels in placenta. Existing as four alternatively spliced isoforms, ITI-H5 is composed of multipolypeptides, possibly including one heavy chain containing potential calcium-binding sites and two light chains. ITI-H5 encodes a heavy chain component of one of the inter- α -trypsin inhibitor family members and participates in extracellular matrix stabilization, tumor metastasis prevention and plasma serine protease inhibition. ITI-H5 consists of two conserved ITIH domains: a vault inter- α -trypsin (VIT) domain and a von Willebrand type A (VWA) domain. Decreased expression of ITI-H5 may be involved in various tumor and breast cancer development.

REFERENCES

1. Salier, J.P., et al. 1987. Isolation and characterization of cDNAs encoding the heavy chain of human inter- α -trypsin inhibitor (I α TI): unambiguous evidence for multipolypeptide chain structure of I α TI. *Proc. Natl. Acad. Sci. USA* 84: 8272-8276.
2. Himmelfarb, M., et al. 2004. ITIH5, a novel member of the inter- α -trypsin inhibitor heavy chain family is downregulated in breast cancer. *Cancer Lett.* 204: 69-77.
3. Dahl, E., et al. 2005. Systematic identification and molecular characterization of genes differentially expressed in breast and ovarian cancer. *J. Pathol.* 205: 21-28.
4. Werbowetski-Ogilvie, T.E., et al. 2006. Isolation of a natural inhibitor of human malignant glial cell invasion: inter α -trypsin inhibitor heavy chain 2. *Cancer Res.* 66: 1464-1472.
5. Veeck, J., et al. 2008. The extracellular matrix protein ITIH5 is a novel prognostic marker in invasive node-negative breast cancer and its aberrant expression is caused by promoter hypermethylation. *Oncogene* 27: 865-876.
6. Veeck, J., et al. 2008. Novel prognostic marker in invasive breast cancer. ITIH5 expression is abrogated by aberrant promoter methylation. *Pathologie* 29: 338-346.

CHROMOSOMAL LOCATION

Genetic locus: Itih5 (mouse) mapping to 2 A1.

PRODUCT

ITI-H5 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ITI-H5 shRNA Plasmid (m): sc-146309-SH and ITI-H5 shRNA (m) Lentiviral Particles: sc-146309-V as alternate gene silencing products.

For independent verification of ITI-H5 (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-146309A, sc-146309B and sc-146309C.

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

ITI-H5 siRNA (m) is recommended for the inhibition of ITI-H5 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 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

ITI-H5 (D-1): sc-390885 is recommended as a control antibody for monitoring of ITI-H5 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 ITI-H5 gene expression knockdown using RT-PCR Primer: ITI-H5 (m)-PR: sc-146309-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.