SANTA CRUZ BIOTECHNOLOGY, INC.

DDT siRNA (h): sc-77103

BACKGROUND

Macrophage migration inhibitory factor, known as MIF or glycosylation-inhibiting factor, is a secreted, homotrimeric, pro-inflammatory cytokine that modulates macrophage and T cell function and is an important regulator of host response to infection. MIF is expressed at sites of inflammation, which suggests that it plays a role in regulating macrophage function in host defense. The only known family member of MIF is D-dopachrome tautomerase (DDT), a protein that is thought to similarly play a role in the inflammation process. DDT is highly expressed in liver with lower levels in other organs, including heart, lung and pancreas. It resides in the cytoplasm as a homotrimer and converts 2-carboxy-2,3-dihyroindole-5, 6-quinone (D-dopachrome) into 5,6-dihydroxyindole. DDT requires the presence of an N-terminal proline residue for catalytic activity and is involved in the biosynthesis of melanin, an antioxidant. In response to liver damage, DDT has been shown to increase protein levels in order to accelerate melanin biosynthesis and protect the liver from oxidative stress.

REFERENCES

1. Weiser, W.Y., et al. 1989. Molecular cloning of a cDNA encoding a human macrophage migration inhibitory factor. Proc. Natl. Acad. Sci. USA 86: 7522-7526.
2. Paralkar, V. and Wistow, G. 1994. Cloning the human gene for macrophage migration inhibitory factor (MIF). Genomics 19: 48-51.
3. Bernhagen, J., et al. 1994. Purification, bioactivity, and secondary structure analysis of mouse and human macrophage migration inhibitory factor (MIF). Biochemistry 33: 14144-14155.
4. Yoshida, H., et al. 1997. NMR characterization of physicochemical properties of rat D-dopachrome tautomerase. Biochem. Mol. Biol. Int. 42: 891-899.
5. Nishihira, J., et al. 1998. Molecular cloning of human D-dopachrome tautomerase cDNA: N-terminal proline is essential for enzyme activation. Biochem. Biophys. Res. Commun. 243: 538-544.
6. Sugimoto, H., et al. 1999. Crystal structure of human D-dopachrome tautomerase, a homologue of macrophage migration inhibitory factor, at 1.54 A resolution. Biochemistry 38: 3268-3279.

CHROMOSOMAL LOCATION

Genetic locus: DDT (human) mapping to 22q11.23.

PRODUCT

DDT siRNA (h) is a target-specific 19-25 nt siRNA 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 DDT shRNA Plasmid (h): sc-77103-SH and DDT shRNA (h) Lentiviral Particles: sc-77103-V as alternate gene silencing products.

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

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

DDT (1G1): sc-517061 is recommended as a control antibody for monitoring of DDT 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 DDT gene expression knockdown using RT-PCR Primer: DDT (h)-PR: sc-77103-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.