

MPO siRNA (m): sc-43942

BACKGROUND

The heme protein myeloperoxidase (MPO) is a major component of azurophilic granules of neutrophils and polymorphonuclear leukocytes. Optimal oxygen-dependent microbicidal activity depends on MPO as the critical enzyme for the generation of hypochlorous acid and other toxic oxygen products. The MPO precursor is synthesized during the promyelocytic stage of myeloid differentiation and is subsequently processed and transported intracellularly to the lysosomes. The precursor undergoes cotranslational N-linked glycosylation to produce a glycoprotein. Glucosidases in the endoplasmic reticulum (ER) or early *cis* Golgi convert the pro-MPO to a form which is sorted into a prelysosomal compartment, which undergoes final proteolytic maturation to native MPO, a pair of heavy-light protomers. In normal neutrophils, MPO is expressed as a dimer. Calreticulin, a calcium-binding protein residing in the ER, interacts specifically with fully glycosylated apopro-MPO. iMPO mRNA is abundant in human promyelocytic HL-60 and mouse myeloid leukemia NFS-60 cells. MPO is expressed at high levels in circulating neutrophils and monocytes but is not detectable in microglia, brain-specific macrophages or normal brain tissue.

REFERENCES

1. Johnson, K.R., et al. 1987. Characterization of cDNA clones for human myeloperoxidase: predicted amino acid sequence and evidence for multiple mRNA species. *Nucleic Acids Res.* 15: 2013-2028.
2. Nauseef, W.M. 1987. Posttranslational processing of a human myeloid lysosomal protein, myeloperoxidase. *Blood* 70: 1143-1150.
3. Morishita, K., et al. 1987. Molecular cloning and characterization of cDNA for human myeloperoxidase. *J. Biol. Chem.* 262: 3844-3851.
4. Nauseef, W.M., et al. 1988. Biosynthesis and processing of myeloperoxidase a marker for myeloid cell differentiation. *Eur. J. Haematol.* 40: 97-110.
5. Homma, T., et al. 1989. Preparation and characterization of monoclonal antibodies against human myeloperoxidase. *Arch. Biochem. Biophys.* 273: 189-196.
6. Zuurbier, K.W., et al. 1992. Human hemi-myeloperoxidase. Initial chlorinating activity at neutral pH, compound II and III formation, and stability towards hypochlorous acid and high temperature. *Eur. J. Biochem.* 205: 737-742.

CHROMOSOMAL LOCATION

Genetic locus: Mpo (mouse) mapping to 11 C.

PRODUCT

MPO 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 MPO shRNA Plasmid (m): sc-43942-SH and MPO shRNA (m) Lentiviral Particles: sc-43942-V as alternate gene silencing products.

For independent verification of MPO (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-43942A, sc-43942B and sc-43942C.

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

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

MPO light chain (C-3): sc-390109 is recommended as a control antibody for monitoring of MPO 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 MPO gene expression knockdown using RT-PCR Primer: MPO (m)-PR: sc-43942-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.