

MITF siRNA (m): sc-35935

BACKGROUND

MITF (microphthalmia-associated transcription factor) is a melanocytic nuclear protein that contains basic helix-loop-helix (HLH) and leucine zipper (LZ) domains. These protein motifs are frequently observed in other transcription factors and are particularly common to members of the Myc family. MITF can directly associate with DNA as a homodimer and is required for the development and differentiation of melanocytes. Its expression is upregulated by cAMP and cAMP-dependent pathways. MITF activates several different gene promoters by binding to their E-boxes. Tyrosinase, TRP1 and TRP2 are pigment synthesis genes activated by MITF. When MITF is phosphorylated on Ser 73 (via the MAPK pathway), it associates with co-activators of the p300/CBP family and enhances transcription. MITF has several isoforms including MITF-M which is specifically expressed in melanocytes. In MITF-deficient mice there is a complete absence of melanocytes.

REFERENCES

1. Beckmann, H., et al. 1990. TFE3: a helix-loop-helix protein that activates transcription through the immunoglobulin enhancer μ E3 motif. *Genes Dev.* 4: 167-179.
2. Fisher, D.E., et al. 1991. TFEB has DNA-binding and oligomerization properties of a unique helix-loop-helix/leucine-zipper family. *Genes Dev.* 5: 2342-2352.
3. Kerkhoff, E., et al. 1991. Sequence-specific DNA binding by Myc proteins. *Proc. Natl. Acad. Sci. USA* 88: 4323-4327.
4. Artandi, S.E., et al. 1994. The basic helix-loop-helix-zipper domain of TFE3 mediates enhancer-promoter interaction. *Mol. Cell. Biol.* 14: 7704-7716.

CHROMOSOMAL LOCATION

Genetic locus: Mitf (mouse) mapping to 6 D3.

PRODUCT

MITF 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 MITF shRNA Plasmid (m): sc-35935-SH and MITF shRNA (m) Lentiviral Particles: sc-35935-V as alternate gene silencing products.

For independent verification of MITF (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-35935A, sc-35935B and sc-35935C.

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

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

MITF (D-9): sc-515925 is recommended as a control antibody for monitoring of MITF 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 λ BP-HRP: sc-516132 or m-IgG λ BP-HRP (Cruz Marker): sc-516132-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 λ BP-FITC: sc-516185 or m-IgG λ BP-PE: sc-516186 (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 MITF gene expression knockdown using RT-PCR Primer: MITF (m)-PR: sc-35935-PR (20 μ l, 509 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Chiu, C.S., et al. 2018. Exploiting Honokiol-induced ER stress CHOP activation inhibits the growth and metastasis of melanoma by suppressing the MITF and β -catenin pathways. *Cancer Lett.* 442: 113-125.
2. Tsang, T.F., et al. 2019. *Gynostemma pentaphyllum* saponins induce melanogenesis and activate cAMP/PKA and Wnt/ β -catenin signaling pathways. *Phytomedicine* 60: 153008.
3. Mehdi, A., et al. 2023. S-adenosylmethionine blocks tumorigenesis and with immune checkpoint inhibitor enhances anti-cancer efficacy against BRAF mutant and wildtype melanomas. *Neoplasia* 36: 100874.

RESEARCH USE

For research use only, not for use in diagnostic procedures.