

Anti-MiTF antibody, rabbit serum, ChIP grade

73-107 100 μ l

Storage: Shipped at 4°C, and aliquot and store at -20°C or below

Reactivity: Specific to human, mouse, chicken and Xenopus Mitf. This antibody recognizes MiTF-M, MiTF-A, MiTF-C, MiTF-H and other MiTF isoforms.

Immunogen: Recombinant full-size human Mitf (isoform M) protein with His tag

Validation: Specificity of the antibody reactivity to Mitf was validated with siRNA

Applications:

1. Western blotting (1/1,000-1/3,000)
2. Immunohistochemistry (1/300 ~ 1/1,000).
3. Immunofluorescence staining
4. ChIP (1/200)

Form: Antiserum added with 0.05% sodium azide

Background: **Mitf** (Microphthalmia-associated transcription factor) is a transcription factor that contains both basic helix-loop-helix and leucine zipper structural features. It plays a critical role in the differentiation of various cell types such as neural crest-derived melanocytes, mast cells, osteoclasts and optic cup-derived retinal pigment epithelium. Mutations in **Mitf** cause auditory-pigmentary syndromes, such as Waardenburg syndrome type 2 and Tietz syndrome. Alternatively spliced transcript variants encoding different isoforms have been identified.

Data Link: UniProtKB/Swiss-Prot human: [O75030](#) (MITF_HUMAN), mouse: [Q08874](#) (MITF_MOUSE), chicken: [O73871](#) (O73871_CHICK), Xenopus: [A4IID0](#) (A4IID0_XENTR), OMIM (human): [156845](#)

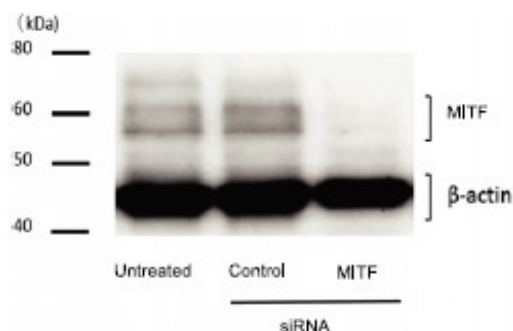


Fig.1 Western blot of Mitf in human melanoma cells.

Untreated: whole cell lysate

Control: Lysate of cells treated with control siRNA.

Mitf: Lysate of cells treated with siRNA against Mitf mRNA.

Anti-Mitf antibody was diluted at 1/1,000 in Can-Get-Signal Immunoreaction Enhancer Solution (Toyobo, Tokyo). Upper band of Mitf is phosphorylated form of Mitf-M. The lower band is Mitf-M isoform.

Anti- β actin antibody was as a loading control.

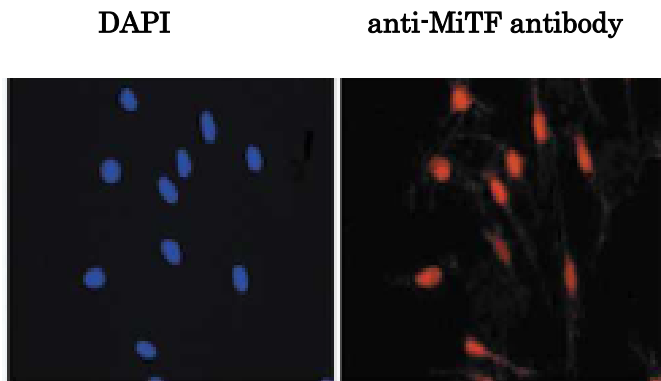


Fig.2 Immunofluorescence staining of melanocytes.

Mouse primary melanocytes 6 weeks after explantation was processed for immunofluorescence microscopy using anti-MiTF antibody at 1/500 dilution and DAPI

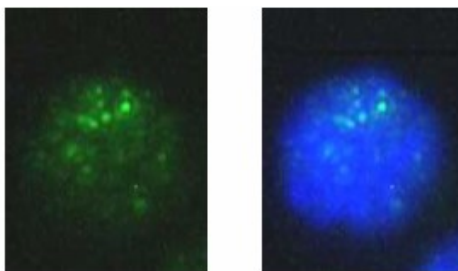


Fig.3 Immunofluorescence staining of mouse melanoma cell.

Mouse melanoma B 16 cells are fixed with 4% PFA, permeabilized with 0.25 % Triton X-100. Anti-Mitf antibody was used at 1/1,000 dilution and as the secondary antibody, goat anti-rabbit IgG antibody (Alexa Fluor 488 conjugated) was used at 1/1,000 dilution.

Left: Immunofluorescence staining with the antibody
Right: Merged image with nucleus stained with DAPI.

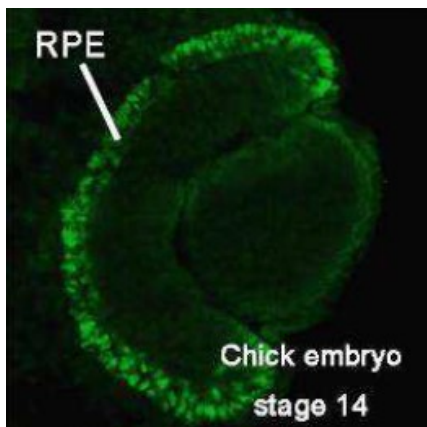


Fig.4 Immunohistochemical staining of Mitf in chick embryo at stage 14.

Embryo was fixed with paraformaldehyde and embedded in OCT compound and sectioned with a cryostat at 8 μ m. Anti-MiTF antibody was used at 1/300 dilution. As second antibody, Alexa 488 conjugated anti-rabbit IgG was used.

At stage 14, Mitf protein is detected throughout the RPE (Retinal Pigment Epithelium).

References: This antibody has been used in the following publications.

1. Osawa M et al. Molecular characterization of melanocyte stem cells in their niche. *Development* **132**: 5589-5599 (2005) PMID: [16314490](https://pubmed.ncbi.nlm.nih.gov/16314490/) IHC-Fr (ms)

2. Delmas V et al. β -Catenin induces immortalization of melanocytes by suppressing p16INK4a expression and co-operates with N-Ras in melanoma development. *Genes Dev* **21**: 2923-2935 (2007) PMID: [18006687](#) **IF (ms)**
3. Yonetani S. et al. *In Vitro* Expansion of Immature Melanoblasts and their Ability to Repopulate Melanocyte Stem Cells in the Hair Follicle. *J Invest Dermatol.* 2008 Feb;128(2):408-20. PMID:17657242. **IHC-Fr (ms)**
4. Inoue-Narita T. et al. Pten deficiency in melanocytes results in resistance to hair graying and susceptibility to carcinogen-induced melanomagenesis. *Cancer Res.* 2008 Jul 15;68(14):5760-8. PMID:[18632629](#) **WB (ms)**
5. Tsukiji N et al. Mitf functions as an in ovo regulator for cell differentiation and proliferation during development of the chick RPE. *Dev Biol* **326**: 335-346 (2009) PMID: [19100253](#) **IHC-Fr, ChIP, (chicken)**
6. Ohba K. et al. Microphthalmia-associated transcription factor is expressed in projection neurons of the mouse olfactory bulb. *Genes Cells.* 2015 Dec;20 (12): 1088-102. PMID:26522736. **WB, IHC-P (ms)**
7. Takeda K. et al. Regional Fluctuation in the Functional Consequence of LINE-1 Insertion in the Mitf Gene: The Black Spotting Phenotype Arisen from the Mitfmi-bw Mouse Lacking Melanocytes. *PLoS One.* 2016 Mar 1;11(3): PMID:26930598. **IHC-P (ms)**
8. Ohba K. et al. Microphthalmia-associated transcription factor ensures the elongation of axons and dendrites in the mouse frontal cortex. *Genes Cells.* 2016 Dec;21(12):1365-1379. PMID:27859996 **IHC-P (ms)**