

## Anti-MITF antibody, rabbit serum, ChIP grade

73-107 100  $\mu$ l

**Shipping and Storage:** Shipped at 4°C or -20°C, and store at -20°C.

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

**Form:** Antiserum added with 0.05% sodium azide

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

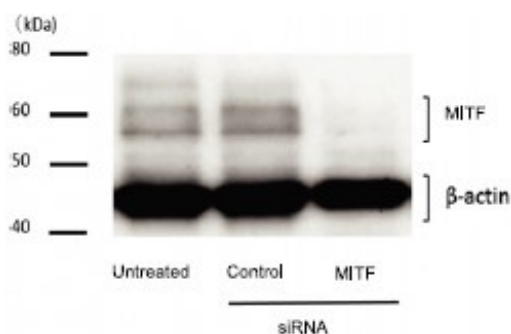
**Validation:** Specificity of the antibody reactivity to Mitf was validated with siRNA

### Applications:

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

**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: [Q75030](#) (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.

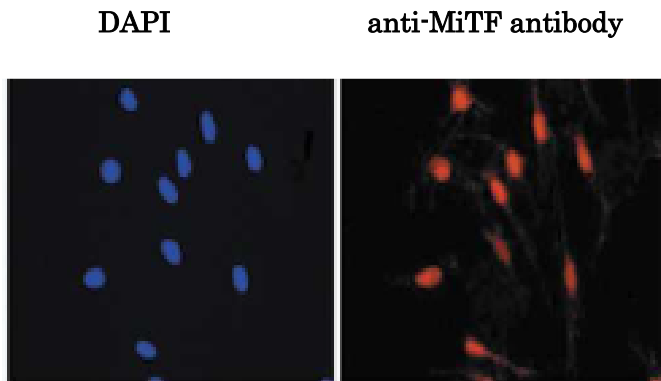
Untreated: whole cell lysate

Control: Lysate of cells treated with control siRNA.

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

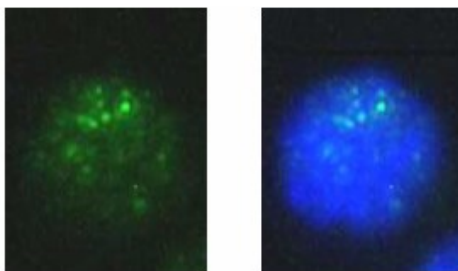
Anti-Mitf antibody was diluted at 1/1,000 in Can-Get-Signal Immunoreaction Enhancer Solution (Toyobo, Tokyo). Upper band of Mitf is phosphorylated form of Mitf-M. The lower band is Mitf-M isoform.

Anti- $\beta$  actin antibody was as a loading control.



**Fig.2 Immunofluorescence staining of melanocytes.**

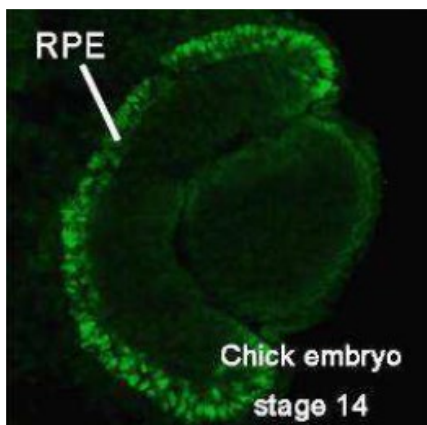
Mouse primary melanocytes 6 weeks after explantation was processed for immunofluorescence microscopy using anti-MiTF antibody at 1/500 dilution and DAPI



**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  $\mu$ 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..  $\beta$ -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)**

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