

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

73-108 100 μg

**Shipping and Storage**: Shipped at  $4^{\circ}$ C or  $-20^{\circ}$ C, and store at  $-20^{\circ}$ C.

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

Form: 1.0 mg/ml IgG fraction of antiserum in PBS- with 50% glycerol

**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

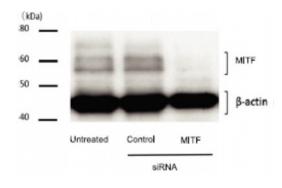
#### Applications:

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

**Backgroud: 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: <u>O75030</u> (MITF\_HUMAN), mouse: <u>Q08874</u> (MITF\_MOUSE), chicken: <u>O73871</u> (O73871\_CHICK), Xenopus: <u>A4IID0</u> (A4IID0\_XENTR), OMIM (human): <u>156845</u>

Related Products: #73-107 Anti-MITF antibody, rabbit serum



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

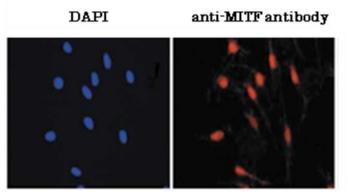
Untreared:whole cell lysate

Control: Lysate of cells treated with control siRNA.

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

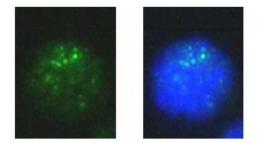
Anti-Mitf antibody was diluted at 1/1,000 in Can-Get-Signal Immunoreaction Enhanceer Solution (Toyobo,Tokyo). Upper band of Mitf is phosphorylated form of Mitf-M. The lower band is Mitf-M isoform. Anti- $\beta$  actin antibody was as a loading control.





# Fig.2 Immunofluorescence staining of melanocytes.

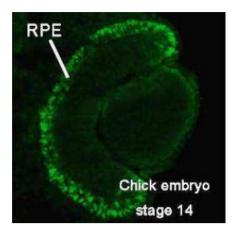
Mouse primary melanocytes 6 weeks after explantation was processed for immunofluorescence microscopy using anti-MiTF antibody at 1/500 dilution and DAPI



## Fig.3 Immunofluoresce 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 conjugted) was used at 1/1,000 dilution.

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



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

Embrio was fixed with paraformaldehyde and embedded in OCT compound and sectioned with a cryostat at 8 µm. Anti-MiTF antibody was used at 1/300 dilution. As second antibody, Alexa 488 conjugated anti-rabbit IgG was used. At stage 14, Mitif protein is detected throughout the RPE (Retinal Pigment Epithelium).

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

- Osawa M et al. Molecular characterization of melanocyte stem cells in their niche. Development 132: 5589-5599 (2005) PMID: <u>16314490</u> IHC-Fr (ms)
- 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. <u>J Invest Dermatol.</u> 2008 Feb;128(2):408-20.

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#### PMID:17657242. IHC-Fr (ms)

- Inoue-Narita T. et al. Pten deficiency in melanocytes results in resistance to hair graying and susceptibility to carcinogen-induced melanomagenesis. <u>Cancer Res.</u> 2008 Jul 15;68(14):5760-8. PMID:<u>18632629</u> WB (ms)
- 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: <u>19100253</u> IHC-Fr, ChIP, (chicken)
- 6. Ohba K. et al. Microphthalmia-associated transcription factor is expressed in
- projection neurons of the mouse olfactory bulb.<u>Genes Cells.</u> 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. <u>PLoS One.</u> 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. <u>Genes Cells.</u> 2016 Dec;21(12):1365-1379. PMID:27859996 **IHC-P (ms)**

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