Anti-MiTF Antibody [JF100-01] - BSA and Azide free

HA750362

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, FC
Molecular Wt:	Predicted band size: 59 kDa
Clone number:	JF100-01

Description: 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 Ser73 (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.

Immunogen: Recombinant protein within Human MiTF aa 353-520 / 526.

Positive control: SK-MEL-28 cell lysate, HeLa cell lysate, A375 cell lysate, A172 cell lysate, B16-F1 cell lysate, PC-12 cell lysate, Hela, A431, NIH/3T3, SW480.

Subcellular location: Nucleus, Cytoplasm.

Database links: SwissProt: O75030 Human | Q08874 Mouse | O88368 Rat

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:50-1:100
FC	1:50-1:100

Storage Buffer: 1*PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

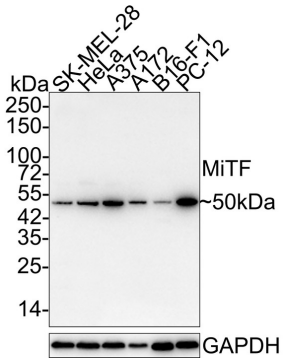

Fig1: Western blot analysis of MiTF on different lysates with Rabbit anti-MiTF antibody (HA750362) at 1/1,000 dilution.

Lane 1: SK-MEL-28 cell lysate (20 µg/Lane)
Lane 2: HeLa cell lysate (20 µg/Lane)
Lane 3: A375 cell lysate (20 µg/Lane)
Lane 4: A172 cell lysate (20 µg/Lane)
Lane 5: B16-F1 cell lysate (20 µg/Lane)
Lane 6: PC-12 cell lysate (20 µg/Lane)

Predicted band size: 59 kDa
Observed band size: 50 kDa

Exposure time: 5 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750362) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

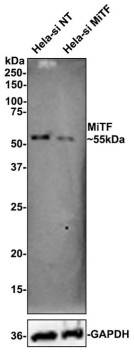

Fig2: Western blot analysis of MiTF on different lysates with Rabbit anti-MiTF antibody (HA750362) at 1/1,000 dilution.

Lane 1: Hela-si NT cell lysate (10 µg/Lane)
Lane 2: Hela-si MiTF cell lysate (10 µg/Lane)

Predicted band size: 59 kDa
Observed band size: 55 kDa

Exposure time: 3 minutes 26 seconds;

4-20% SDS-PAGE gel.

ET1702-86 was shown to specifically react with MiTF in Hela-si NT cells. Weakened band was observed when Hela-si MiTF sample was tested. Hela-si NT and Hela-si MiTF samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1702-86, 1/1,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% BSA at 4 ℃ overnight. Goat Anti-rabbit IgG-HRP Secondary Antibody (HA1001) at 1/100,000 dilution was used for 1 hour at room temperature.

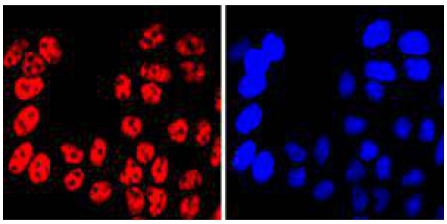

Fig3: ICC staining of MiTF in Hela cells (red). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (HA750362, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®594 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

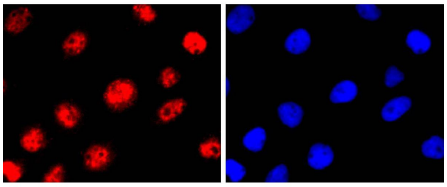

Fig4: ICC staining of MiTF in A431 cells (red). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (HA750362, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®594 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

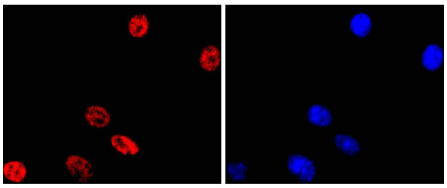

Fig5: ICC staining of MiTF in NIH/3T3 cells (red). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (HA750362, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®594 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

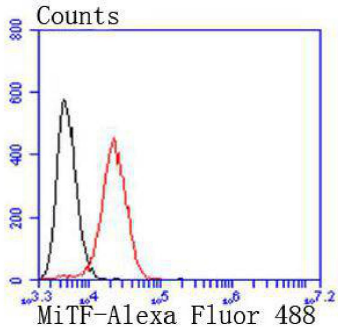

Fig6: Flow cytometric analysis of MiTF was done on SW480 cells. The cells were fixed, permeabilized and stained with the primary antibody (HA750362, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1,000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Stemig M et al. Deletion of histone deacetylase 7 in osteoclasts decreases bone mass in mice by interactions with MITF. PLoS One 10:e0123843 (2015).
2. Sohn EH et al. Allogenic iPSC-derived RPE cell transplants induce immune response in pigs: a pilot study. Sci Rep 5:11791 (2015).