


Product datasheet

Anti-MiTF antibody [D5] ab3201

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Overview

Product name	Anti-MiTF antibody [D5]
Description	Mouse monoclonal [D5] to MiTF
Host species	Mouse
Tested applications	Suitable for: WB, Flow Cyt (Intra), ICC
Species reactivity	Reacts with: Human Predicted to work with: Mouse, Dog  Does not react with: Rat
Immunogen	Fusion protein. This information is proprietary to Abcam and/or its suppliers.
Epitope	N-terminal
Positive control	Flow Cyt (Intra): Malme-3 cells. ICC: Malme-3M cells. WB: HeLa whole cell and nuclear lysate.
General notes	<p>This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com.</p> <p>The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.</p> <p>If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
Purity	Protein G purified
Clonality	Monoclonal
Clone number	D5

Isotype	IgG1
Light chain type	kappa

Applications

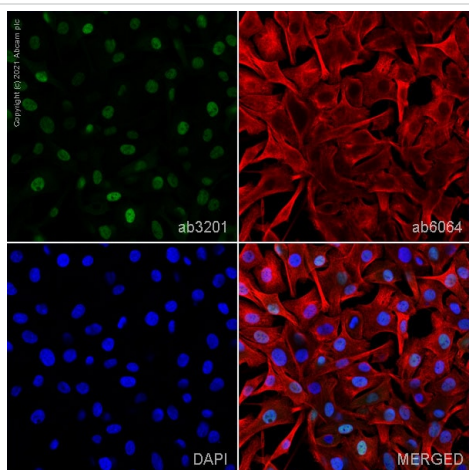
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab3201 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 59 kDa.
Flow Cyt (Intra)		Use 1 µg for 10 ⁶ cells. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
ICC		Use a concentration of 1 µg/ml.

Target

Function	Transcription factor for tyrosinase and tyrosinase-related protein 1. Binds to a symmetrical DNA sequence (E-boxes) (5'-CACGTG-3') found in the tyrosinase promoter. Plays a critical role in the differentiation of various cell types as neural crest-derived melanocytes, mast cells, osteoclasts and optic cup-derived retinal pigment epithelium.
Tissue specificity	Isoform M is exclusively expressed in melanocytes and melanoma cells. Isoform A and isoform H are widely expressed in many cell types including melanocytes and retinal pigment epithelium (RPE). Isoform C is expressed in many cell types including RPE but not in melanocyte-lineage cells.
Involvement in disease	Defects in MITF are the cause of Waardenburg syndrome type 2A (WS2A) [MIM:193510]. It is a dominant inherited disorder characterized by sensorineural hearing loss and patches of depigmentation. The features show variable expression and penetrance. Defects in MITF are a cause of Waardenburg syndrome type 2 with ocular albinism (WS2-OA) [MIM:103470]. It is an ocular albinism with sensorineural deafness. Defects in MITF are the cause of Tietz syndrome (TIETZS) [MIM:103500]. It is an autosomal dominant disorder characterized by generalized hypopigmentation and profound, congenital, bilateral deafness. Penetrance is complete.
Sequence similarities	Belongs to the MiT/TFE family. Contains 1 basic helix-loop-helix (bHLH) domain.
Post-translational modifications	Phosphorylation at Ser-405 significantly enhances the ability to bind the tyrosinase promoter.
Cellular localization	Nucleus.

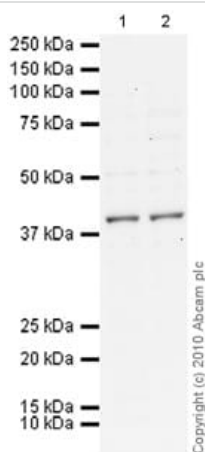
Images



Immunocytochemistry - Anti-MiTF antibody [D5] (ab3201)

ab3201 staining MiTF in Malme-3M cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab3201 at 1µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with **ab150117**, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150080**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Western blot - Anti-MiTF antibody [D5] (ab3201)

All lanes : Anti-MiTF antibody [D5] (ab3201) at 5 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) whole cell lysate

Lane 2 : HeLa nuclear lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

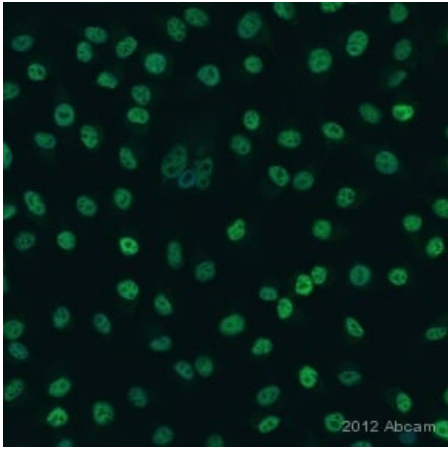
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 59 kDa

Observed band size: 40 kDa

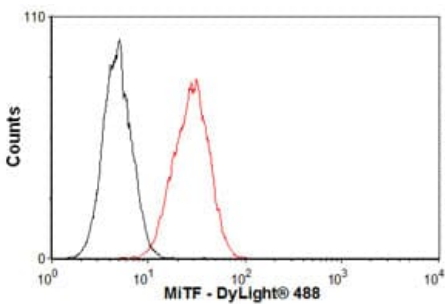
Exposure time: 10 minutes



Immunocytochemistry - Anti-MiTF antibody [D5] (ab3201)

This image is courtesy of an anonymous Abreview

ab3201 staining MiTF in the SK-MEL-28 (Human cutaneous melanoma cell line) cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with Paraformaldehyde and permeabilized with Triton X-100 0.5% in PBS. Samples were incubated with primary antibody (1/100) for 24 hours at 4°C. An Alexa Fluor®488-conjugated Goat anti-mouse IgG polyclonal(1/1000) was used as the secondary antibody. SK-MEL-28 cells was grown on chamber slide. MiTF was found to strictly localised in the nucleus. Cells were counter-stained with DAPI.



Flow Cytometry (Intracellular) - Anti-MiTF antibody [D5] (ab3201)

Overlay histogram showing Malme-3 (Human skin fibroblast cell line) cells stained with ab3201 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab3201, 1µg/1x10⁶ cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 2µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed.

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