abcam

Product datasheet

Anti-mtTFA antibody [RM1035] ab307302

Recombinant RabMAb

★★★★ 1 Abreviews 10 Images

Overview

Product name Anti-mtTFA antibody [RM1035]

Description Rabbit recombinant multiclonal [RM1035] to mtTFA

Host species Rahhit

Specificity IHC-P is not suitable for testing mouse and rat samples.

Tested applications Suitable for: WB, IHC-P, ICC/IF, Flow Cyt, IP

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen This product was produced with the following immunogens:

Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HepG2 mitochondria / non-mitochondria fraction, MCF7, HEK-293, C2C12, NIH/3T3, and

C6 whole cell lysates; mouse and rat heart tissue lysates. IHC-P: Human colon and lung

carcinoma FFPE tissue sections. ICC/IF: U-2 OS and C2C12 cells. Flow Cyt: MCF7 and C2C12

cells. IP: MCF7 and C2C12 whole cell lysates.

General notes Our RabMAb® technology is a patented hybridoma-based technology for making rabbit

monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

Recombinant multiclonals are a mixture of recombinant antibodies co-expressed from a library of

heavy and light chains.

Recombinant multiclonal antibodies offer the sensitivity of polyclonal antibodies by recognising

multiple epitopes, along with consistency of a recombinant antibody.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Recombinant Multiclonal

Clone number RM1035

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab307302 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	*** <u>*</u>	1/1000. Detects a band of approximately 25 kDa.
IHC-P		1/1000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. IHC-P is not suitable for testing mouse and rat samples
ICC/IF		1/100.
Flow Cyt		1/500.
IP		1/30.

Target

Function

Binds to the mitochondrial light strand promoter and functions in mitochondrial transcription regulation. Required for accurate and efficient promoter recognition by the mitochondrial RNA polymerase. Promotes transcription initiation from the HSP1 and the light strand promoter by binding immediately upstream of transcriptional start sites. Is able to unwind and bend DNA. Required for maintenance of normal levels of mitochondrial DNA. May play a role in organizing and compacting mitochondrial DNA. target DNA. Interacts with TFB1M and TFB2M.

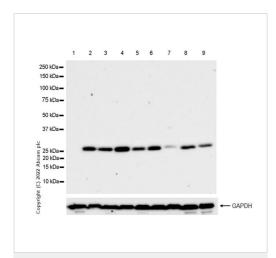
Sequence similarities

Contains 2 HMG box DNA-binding domains.

Cellular localization

Mitochondrion.

Images



Western blot - Anti-mtTFA antibody [RM1035] (ab307302)

All lanes : Anti-mtTFA antibody [RM1035] (ab307302) at 1/1000 dilution

Lane 1: HepG2 (human hepatocellar carcinoma epithelial cell) non-mitochondrial fraction

Lane 2: HepG2 mitochondria fraction

Lane 3 : MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysate

Lane 4 : HEK-293 (human embryonic kidney epithelial cell) whole cell lysate

Lane 5: C2C12 (mouse myoblast) whole cell lysate

Lane 6: NIH/3T3 (mouse embryonic fibroblast) whole cell lysate

Lane 7: C6 (rat glial tumor glial cell) whole cell lysate

Lane 8 : Mouse heart lysate

Lane 9 : Rat heart lysate

Lysates/proteins at 20 µg per lane.

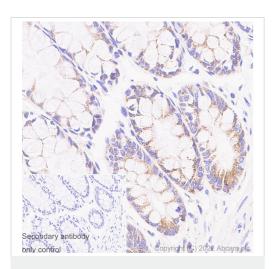
Secondary

All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (<u>ab97051</u>) at 1/100000 dilution

Observed band size: 25 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

Exposure time: 70 seconds

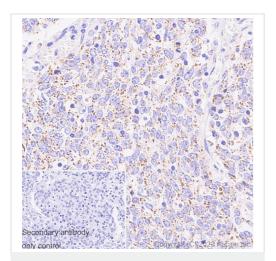


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-mtTFA antibody
[RM1035] (ab307302)

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling mtTFA with AB307302 at 1/1000 (0.54 ug/ml) followed by ready to use Leica DS9800 (Bond[®] Polymer Refine Detection) kit. Positive staining on mitochondria in human colon. The section was incubated with ab307302 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND[®] RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is ready to use Leica DS9800 (Bond[®] Polymer Refine Detection) kit.

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins

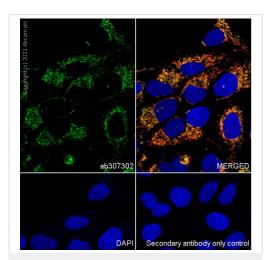


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-mtTFA antibody
[RM1035] (ab307302)

Immunohistochemical analysis of paraffin-embedded human lung carcinoma tissue labeling mtTFA with AB307302 at 1/1000 (0.54 µg/ml) followed by ready to use Leica DS9800 (Bond® Polymer Refine Detection) kit. Positive staining on mitochondria in human lung carcinoma. The section was incubated with ab307302 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is ready to use Leica DS9800 (Bond[®] Polymer Refine Detection) kit.

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins



Immunocytochemistry/ Immunofluorescence - AntimtTFA antibody [RM1035] (ab307302)

ab307302 MERGED

DAPI Secondary antibody only control

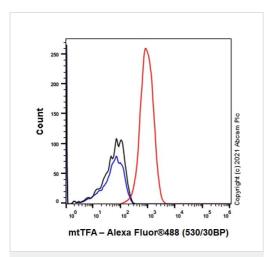
Immunocytochemistry/ Immunofluorescence - AntimtTFA antibody [RM1035] (ab307302)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized U-2 OS (human bone osteosarcoma epithelial cell) cells labeling mtTFA with AB307302 at 1/100 (5.4 μ g/ml) dilution, followed by <u>ab150081</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) preadsorbed antibody at 1/1000 (2 μ g/ml) dilution (Green). Confocal image showing mitochondrial staining in U-2 OS cell line. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). <u>ab197491</u> Anti-COX IV Mouse monoclonal antibody (Alexa Fluor[®] 647) - Mitochondrial Marker was used to counterstain tubulin at 1/200 dilution (2.5 μ g/ml) dilution (Red). The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) preadsorbed at 1/1000 (2 µg/ml) dilution.

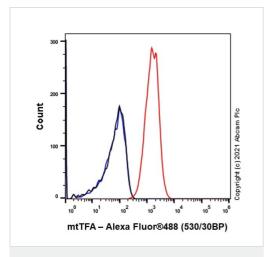
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized C2C12 (mouse myoblasts myoblast) cells labeling mtTFA with AB307302 at 1/100 (5.4 μg/ml) dilution, followed by <u>ab150081</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) preadsorbed antibody at 1/1000 (2 μg/ml) dilution (Green). Confocal image showing cytoplasmic staining in C2C12 cell line. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). <u>ab195889</u> Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor[®] 594) was used to counterstain tubulin at 1/200 dilution (2.5 μg/ml) dilution (Red). The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150081</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) preadsorbed at 1/1000 (2 μ g/ml) dilution.



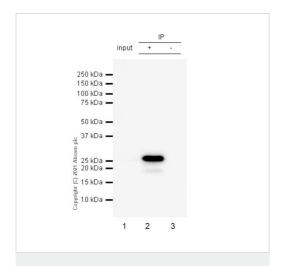
Flow Cytometry - Anti-mtTFA antibody [RM1035] (ab307302)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized MCF7 (human breast adenocarcinoma epithelial cell) cells labeling mtTFA with AB307302 at 1/500 dilution (0.1µg) (Red) compared with a Rabbit monoclonal lgG (ab172730) (Black) isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit lgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody.

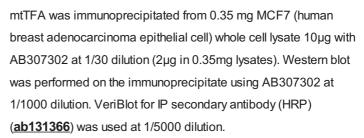


Flow Cytometry - Anti-mtTFA antibody [RM1035] (ab307302)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized C2C12 (mouse myoblast) cells labeling mtTFA with AB307302 at 1/500 dilution (0.1µg) (Red) compared with a Rabbit monoclonal lgG (ab172730) (Black) isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit lgG (Alexa Fluor[®] 488, ab150081) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-mtTFA antibody [RM1035] (ab307302)



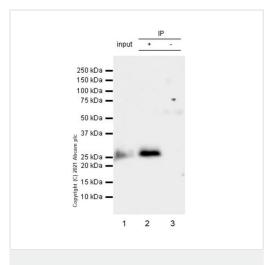
Lane 1: MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysate 10µg

Lane 2: AB307302 IP in MCF7 whole cell lysate

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab307302 in MCF7 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 8 seconds



Immunoprecipitation - Anti-mtTFA antibody [RM1035] (ab307302)

mtTFA was immunoprecipitated from 0.35 mg C2C12 (mouse myoblast) whole cell lysate 10μg with AB307302 at 1/30 dilution (2μg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using AB307302 at 1/1000 dilution. VeriBlot for IP secondary antibody (HRP) (ab131366) was used at 1/5000 dilution.

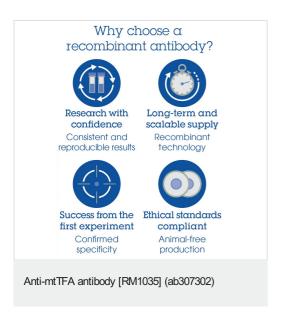
Lane 1: C2C12 (mouse myoblast) whole cell lysate 10µg

Lane 2: AB307302 IP in C2C12 whole cell lysate

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab307302 in C2C12 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 24 seconds



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