# abcam

# Product datasheet

# Anti-TIM44 antibody [EPR16821-26] ab201453



★★★★ 1 Abreviews 8 Images

#### Overview

**Product name** Anti-TIM44 antibody [EPR16821-26]

**Description** Rabbit monoclonal [EPR16821-26] to TIM44

**Host species** Rabbit

**Tested applications** Suitable for: Flow Cyt (Intra), WB, ICC/IF, IP

Species reactivity Reacts with: Rat, Human

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

Positive control WB: K562, Daudi, HepG2, PC-12 and C6 cell lysates; Human fetal liver lysate. ICC/IF: K562 and

HepG2 cells. IP: K562 whole cell lysate.

**General notes** This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information **see here**.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

#### **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 Monoclonal Clone number EPR16821-26

Isotype ΙgG

# **Applications**

# The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab201453 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
Flow Cyt (Intra)		1/500.
WB	<b>★★★★☆ (1)</b>	1/2000. Detects a band of approximately 45 kDa (predicted molecular weight: 51 kDa).
ICC/IF		1/500.
IP		1/120.

# **Target**

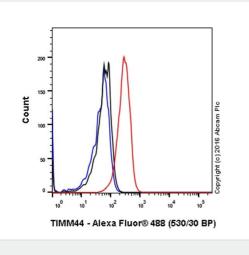
#### Relevance

TIM44 is the essential component of the PAM complex, a complex required for the translocation of transit peptide-containing proteins from the inner membrane into the mitochondrial matrix in an ATP-dependent manner. It recruits mitochondrial HSP70 to drive protein translocation into the matrix using ATP as an energy source.

### **Cellular localization**

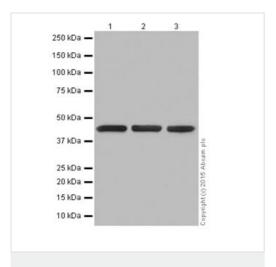
Mitochondrion inner membrane.

### **Images**



Flow Cytometry (Intracellular) - Anti-TIM44 antibody [EPR16821-26] (ab201453)

Intracellular Flow Cytometry analysis of HepG2 (human hepatocellular carcinoma) labelling TIM44 with purified ab201453 at 1/500 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Alexa Fluor<sup>®</sup> 488 goat antirabbit lgG (1/2000) was used as the secondary antibody. Black - lsotype control, rabbit monoclonal lgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Western blot - Anti-TIM44 antibody [EPR16821-26] (ab201453)

**All lanes :** Anti-TIM44 antibody [EPR16821-26] (ab201453) at 1/2000 dilution

**Lane 1 :** K562 (Human chronic myelogenous leukemia cells from bone marrow) cell lysate

Lane 2 : HepG2 (Human liver hepatocellular carcinoma) cell lysate

Lane 3 : Daudi (Human Burkitt's lymphoma cell line) cell lysate

Lysates/proteins at 20 µg per lane.

# Secondary

**All lanes :** Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/1000 dilution

**Predicted band size:** 51 kDa **Observed band size:** 45 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

250 KDa —

150 KDa —

100 KDa —

75 KDa —

50 KDa —

37 KDa —

25 KDa —

20 KDa —

15 KDa —

10 KDa —

10 KDa —

Western blot - Anti-TIM44 antibody [EPR16821-26] (ab201453)

Anti-TIM44 antibody [EPR16821-26] (ab201453) at 1/10000 dilution + Human fetal liver lysate at 10  $\mu g$ 

# **Secondary**

Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG at 1/1000 dilution

Predicted band size: 51 kDa Observed band size: 45 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-TIM44 antibody [EPR16821-26] (ab201453)

**All lanes :** Anti-TIM44 antibody [EPR16821-26] (ab201453) at 1/2000 dilution

Lane 1: C6 (Rat glial tumor cells) cell lysate

Lane 2: PC-12 (Rat adrenal gland pheochromocytoma) cell lysate

Lysates/proteins at 10 µg per lane.

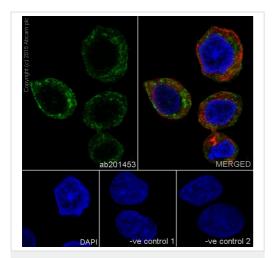
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**Predicted band size:** 51 kDa **Observed band size:** 45 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

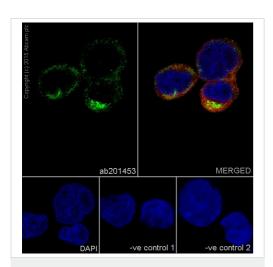


Immunocytochemistry/ Immunofluorescence - Anti-TIM44 antibody [EPR16821-26] (ab201453)

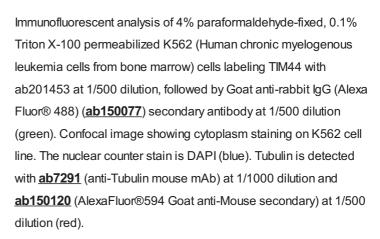
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HepG2 (Human liver hepatocellular carcinoma) cells labeling TIM44 with ab201453 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 dilution (green). Confocal image showing cytoplasm staining on HepG2 cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:

-ve control 1: ab201453 at 1/500 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution. -ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/500 dilution.

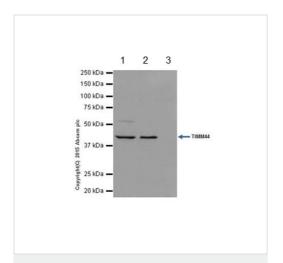


Immunocytochemistry/ Immunofluorescence - Anti-TIM44 antibody [EPR16821-26] (ab201453)



The negative controls are as follows:

-ve control 1: ab201453 at 1/500 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution. -ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/500 dilution.



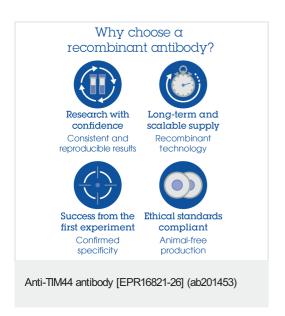
Immunoprecipitation - Anti-TIM44 antibody [EPR16821-26] (ab201453)

TIM44 was immunoprecipitated from 1mg of K562 (Human chronic myelogenous leukemia cells from bone marrow) whole cell lysate with ab201453 at 1/120 dilution. Western blot was performed from the immunoprecipitate using ab201453 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/1500 dilution.

Lane 1: K562 whole cell lysate 10 µg (Input). Lane 2: ab201453 IP in K562 whole cell lysate. Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab201453 in K562 whole cell lysate.

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

Exposure time: 1 second.



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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