abcam

Product datasheet

Anti-TRIM56 antibody [EPR10583] ab154862



Recombinant RabMAb

6 References 12 Images

Overview

Product name Anti-TRIM56 antibody [EPR10583]

Rabbit monoclonal [EPR10583] to TRIM56 **Description**

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, IHC-P, ICC/IF

Species reactivity Reacts with: Human

Immunogen Synthetic peptide within Human TRIM56. The exact sequence is proprietary.

Database link: Q9BRZ2

Positive control HC-P: Human brain, pancreas and colon cancer tissues. WB: HAP1, A549, A375, MCF7 and

HeLa cell lysates. ICC/IF: MCF7 and HeLa cells Flow Cyt (intra): MCF7 cells

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**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

Properties

Liquid **Form**

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

Avoid freeze / thaw cycle.

Storage buffer Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number EPR10583

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab154862 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)		1/20. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For unpurified use at 1/100 - 1/500 dilution.
WB		1/10000 - 1/50000. Predicted molecular weight: 81 kDa.
IHC-P		1/1000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. See IHC antigen retrieval protocols. For unpurifed use at 1/50 -1/100 dilution.
ICC/IF		1/70. For unpurified use at 1/100 - 1/250 dilution.

Target

Function E3 ubiquitin-protein ligase that mediates 'Lys-63'-linked polyubiquitination of TMEM173/STING,

thereby playing a key role in innate immunity. TMEM173/STING 'Lys-63'-linked ubiquitination activates the production of type I interferon IFN-beta following detection of pathogen- and host-

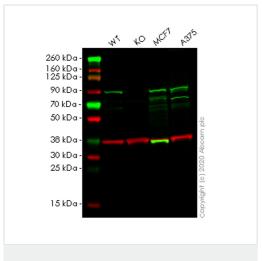
derived double-stranded DNA.

Sequence similarities Belongs to the TRIM/RBCC family.

Contains 2 B box-type zinc fingers. Contains 1 RING-type zinc finger.

Cellular localization Cytoplasm.

Images



Western blot - Anti-TRIM56 antibody [EPR10583] (ab154862)

All lanes : Anti-TRIM56 antibody [EPR10583] (ab154862) at 1/1000 dilution

Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate

Lane 2 : TRIM56 knockout A549 (Human lung carcinoma cell line) whole cell lysate

Lane 3 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

Lane 4 : A-375 (Human malignant melanoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) at 1/10000 dilution

Predicted band size: 81 kDa **Observed band size:** 88 kDa

Lanes 1-4: Merged signal (red and green). Green - ab154862 observed at 88 kDa. Red - loading control <u>ab8245</u> observed at 36 kDa.

ab154862 Anti-TRIM56 antibody [EPR10583] was shown to specifically react with TRIM56 in wild-type A549 cells. Loss of signal was observed when knockout cell line <u>ab267063</u> (knockout cell lysate <u>ab258250</u>) was used. Wild-type and TRIM56 knockout samples were subjected to SDS-PAGE. ab154862 and Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (<u>ab216776</u>) secondary

antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

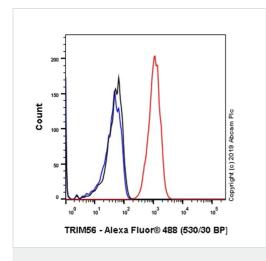
ab154862 MERGED

DAPI

Secondary antibody only control

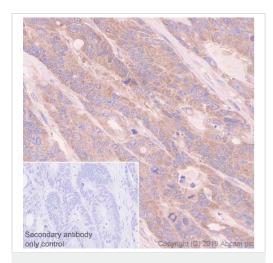
Immunocytochemistry/ Immunofluorescence - Anti-TRIM56 antibody [EPR10583] (ab154862)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling TRIM56 with purified ab154862 at 1/70 dilution (10 μ g/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200 (2.5 μ g/ml) dilution. Goat anti rabbit lgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 μ g/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



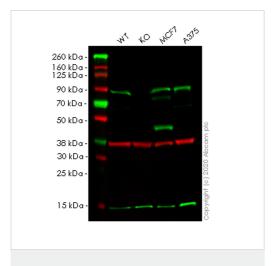
Flow Cytometry (Intracellular) - Anti-TRIM56 antibody [EPR10583] (ab154862)

Intracellular Flow Cytometry analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling TRIM56 with purified ab154862 at 1/70 dilution (10µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor[®] 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TRIM56 antibody
[EPR10583] (ab154862)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human colon cancer tissue sections labeling TRIM56 with purified ab154862 at 1/1000 dilution (0.691 µg/ml). Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Western blot - Anti-TRIM56 antibody [EPR10583] (ab154862)

All lanes : Anti-TRIM56 antibody [EPR10583] (ab154862) at 1/1000 dilution

Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate

Lane 2 : TRIM56 knockout A549 (Human lung carcinoma cell line) whole cell lysate

Lane 3 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

Lane 4: A-375 (Human malignant melanoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 81 kDa
Observed band size: 88 kDa

Lanes 1-4: Merged signal (red and green). Green - ab154862 observed at 88 kDa. Red - loading control <u>ab8245</u> observed at 36 kDa.

ab154862 Anti-TRIM56 antibody [EPR10583] was shown to specifically react with TRIM56 in wild-type A549 cells. Loss of signal

was observed when knockout cell line <u>ab267062</u> (knockout cell lysate <u>ab258249</u>) was used. Wild-type and TRIM56 knockout samples were subjected to SDS-PAGE. ab154862 and Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

1 2

250 kDa —
150 kDa —
100 kDa —
75 kDa —
37 kDa —
25 kDa —
20 kDa —
15 kDa —
10 kDa —
10 kDa —

Western blot - Anti-TRIM56 antibody [EPR10583] (ab154862)

All lanes : Anti-TRIM56 antibody [EPR10583] (ab154862) at 1/50000 dilution (Purified)

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

Lane 2: A375 (Human malignant melanoma epithelial cell) whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 81 kDa **Observed band size:** 81 kDa

260 kDa — 160 kDa — 125 kDa — 90 kDa — 70 kDa — 38 kDa — 30 kDa — 25 kDa —

Western blot - Anti-TRIM56 antibody [EPR10583] (ab154862)

Lane 1: Wild-type HAP1 cell lysate (20 µg)

Lane 2: TRIM56 knockout HAP1 cell lysate (20 µg)

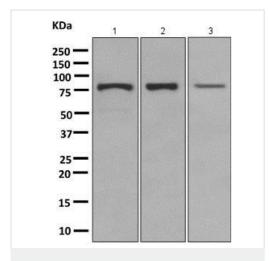
Lane 3: MCF7 cell lysate (20 µg)

Lane 4: A375 cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab154862 observed at 88 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

ab154862 (unpurifed) was shown to recognize TRIM56 when TRIM56 knockout samples were used, along with additional cross-reactive bands. Wild-type and TRIM56 knockout samples were subjected to SDS-PAGE. ab154862 and <u>ab8245</u> (loading control to GAPDH) were both diluted 1/10000 and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-

Mouse IgG H&L (IRDye[®] 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10000 dilution for 1 h at room temperature before imaging.



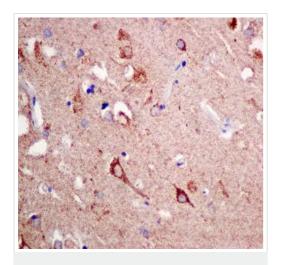
Western blot - Anti-TRIM56 antibody [EPR10583] (ab154862)

All lanes : Anti-TRIM56 antibody [EPR10583] (ab154862) at 1/10000 dilution ((unpurified))

Lane 1 : HeLa cell lysate
Lane 2 : A375 cell lysate
Lane 3 : MCF7 cell lysate

Lysates/proteins at 10 µg per lane.

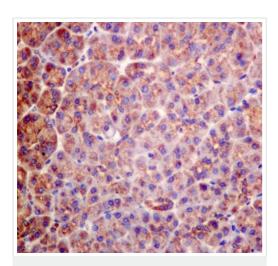
Predicted band size: 81 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TRIM56 antibody
[EPR10583] (ab154862)

Immunohistochemical analysis of paraffin-embedded Human brain tissue labeling TRIM56 with ab154862 (unpurified) at 1/50.

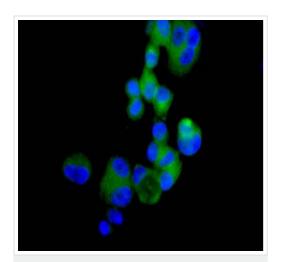
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TRIM56 antibody
[EPR10583] (ab154862)

Immunohistochemical analysis of paraffin-embedded Human pancreas tissue labeling TRIM56 with ab154862 (unpurified) at 1/50.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-TRIM56 antibody [EPR10583] (ab154862)

Immunofluorescent analysis of MCF7 cells labeling TRIM56 with ab154862 (unpurified) at 1/100.



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