abcam

Product datasheet

Anti-TNFAIP3 antibody [EPR2663] ab92324

KO VALIDATED Recombinant RabMAb

★★★★★ 1 Abreviews 31 References 10 Images

Overview

Product name	Anti-TNFAIP3 antibody [EPR2663]
Description	Rabbit monoclonal [EPR2663] to TNFAIP3
Host species	Rabbit
Specificity	**Mouse species is recommended based on WB results, we do not guarantee IHC-P for mouse.**
Tested applications	**Suitable for:** WB, IHC-P **Unsuitable for:** Flow Cyt (Intra) or ICC/IF
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: WEHI-3 treated with TNF (**ab9642**), Jurkat treated with TNF (**ab9642**) + TPA, Jurkat treated with 5ng/ml PMA for 48 hours and then treated with 2Âµg/ml PHA for 48 hours, HeLa, A549 and Daudi cell lysates. IHC-P: Human kidney tissue.
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. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR2663
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab92324 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		1/1000 - 1/5000. Predicted molecular weight: 90 kDa.
IHC-P	★★★★★ **(1)**	1/50 - 1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. **Mouse species is recommended based on WB results, we do not guarantee IHC-P for mouse.**

Application notes Is unsuitable for Flow Cyt (Intra) or ICC/IF.

Target

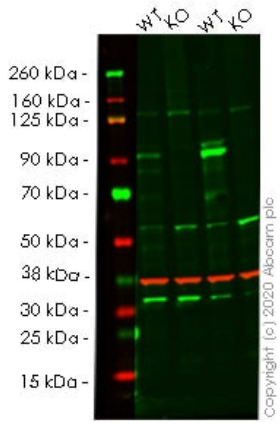
Function Ubiquitin-editing enzyme that contains both ubiquitin ligase and deubiquitinase activities. Essential component of a ubiquitin-editing protein complex, comprising also RNF11, ITCH and TAX1BP1, that ensures the transient nature of inflammatory signaling pathways. Upon TNF stimulation, deubiquitinates 'Lys-63'-polyubiquitin chains on RIPK1 and catalyzes the formation of 'Lys-48'-polyubiquitin chains. This leads to RIPK1 proteasomal degradation and consequently termination of the TNF- or LPS-mediated activation of NF-kappa-B. In vitro able to deubiquitinate both 'Lys-48'- and 'Lys-63' polyubiquitin chains. Inhibitor of programmed cell death. Has a role in the function of the lymphoid system.

Sequence similarities Belongs to the peptidase C64 family.
Contains 7 A20-type zinc fingers.
Contains 1 OTU domain.

Domain The A20-type zinc fingers mediate the ubiquitin ligase activity.
The OTU domain mediates the deubiquitinase activity.

Cellular localization Cytoplasm. Nucleus.

Images



Western blot - Anti-TNFAIP3 antibody [EPR2663] (ab92324)

All lanes : Anti-TNFAIP3 antibody [EPR2663] (ab92324) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : TNFAIP3 knockout A549 cell lysate
Lane 3 : Wild-type HeLa cell lysate
Lane 4 : TNFAIP3 knockout HeLa cell lysate

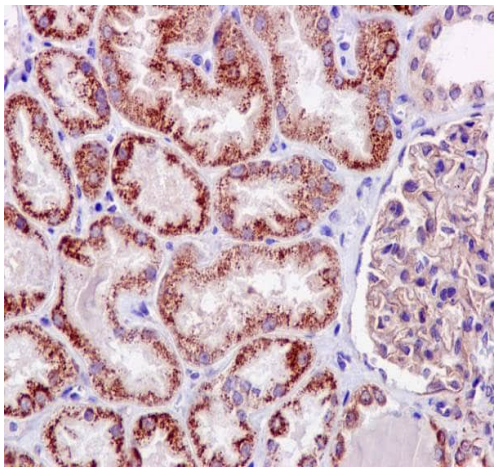
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 90 kDa
Observed band size: 90 kDa

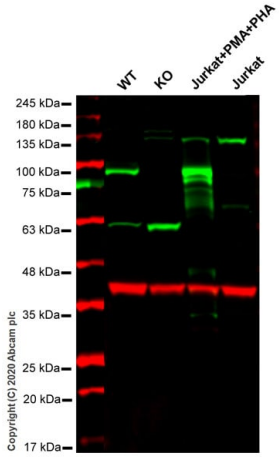
Lanes 1- 4: Merged signal (red and green). Green - ab92324 observed at 90 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab92324 was shown to react with TNFAIP3 in wild-type A549 cells in western blot. Loss of signal was observed when knockout cell line **ab266946** (knockout cell lysate **ab257114**) was used. Wild-type A549 and TNFAIP3 knockout A549 cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab92324 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TNFAIP3 antibody [EPR2663] (ab92324)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney tissue labelling TNFAIP3 with unpurified ab92324 at 1/50. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit IgG was used as the secondary antibody. Counterstained with Hematoxylin.



Western blot - Anti-TNFAIP3 antibody [EPR2663] (ab92324)

All lanes : Anti-TNFAIP3 antibody [EPR2663] (ab92324) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : TNFAIP3 knockout HeLa cell lysate
Lane 3 : Jurkat cell treated with 5ng/ml PMA for 48 hours and then treated with 2µg/ml PHA for 48 hours, whole cell lysate
Lane 4 : Untreated Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

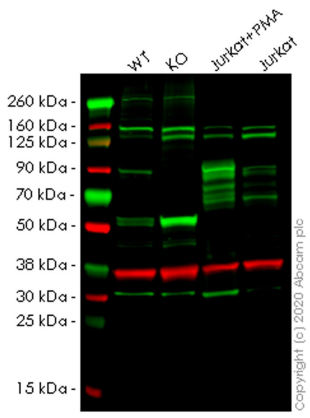
Performed under reducing conditions.

Predicted band size: 90 kDa
Observed band size: 80 kDa

Lanes 1-4: Merged signal (red and green). Green - ab92324 observed at 80 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab92324 Anti-TNFAIP3 antibody [EPR2663] was shown to specifically react with TNFAIP3 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab265983** (knockout

cell lysate **ab257112**) was used. Wild-type and TNFAIP3 knockout samples were subjected to SDS-PAGE. ab92324 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) 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 (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-TNFAIP3 antibody [EPR2663] (ab92324)

All lanes : Anti-TNFAIP3 antibody [EPR2663] (ab92324) at 1/1000 dilution

Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate
Lane 2 : TNFAIP3 knockout A549 (Human lung carcinoma cell line) whole cell lysate
Lane 3 : Jurkat (Human T cell leukemia cell line from peripheral blood) cell treated with 5ng/ml PMA for 48 hours and then treated with 2µg/ml PHA for 48 hours, whole cell lysate
Lane 4 : Untreated Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lysates/proteins at 20 µg per lane.

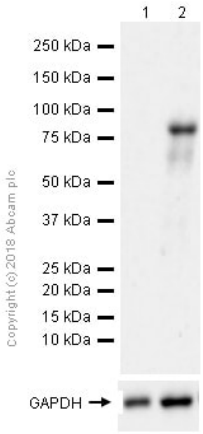
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 90 kDa
Observed band size: 80 kDa

Lanes 1-4: Merged signal (red and green). Green - ab92324 observed at 80 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab92324 Anti-TNFAIP3 antibody [EPR2663] was shown to specifically react with TNFAIP3 in wild-type A549 cells. Loss of signal was observed when knockout cell line **ab266945** (knockout cell lysate **ab257113**) was used. Wild-type and TNFAIP3 knockout samples were subjected to SDS-PAGE. ab92324 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) 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 (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-TNFAIP3 antibody [EPR2663] (ab92324)

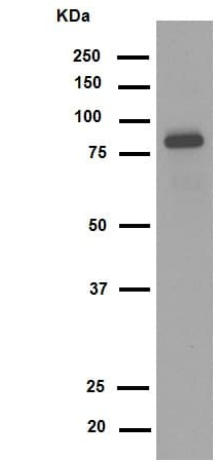
All lanes : Anti-TNFAIP3 antibody [EPR2663] (ab92324) at 1/5000 dilution

Lane 1 : WEHI-3 (Mouse leukemia lymphoblast) whole cell lysate
Lane 2 : WEHI-3 treated with 20 ng/ml TNF alpha (**ab9642**) for 6 h

Lysates/proteins at 20 µg per lane.

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

Predicted band size: 90 kDa
Observed band size: 80 kDa



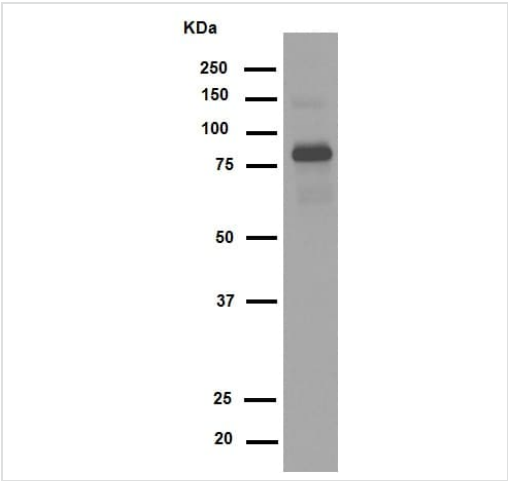
Western blot - Anti-TNFAIP3 antibody [EPR2663] (ab92324)

Anti-TNFAIP3 antibody [EPR2663] (ab92324) at 1/2000 dilution (unpurified) + Jurkat cell lysate - treated with TNF (**ab9642**) and TPA at 10 µg

Secondary
Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 90 kDa
Observed band size: 80 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-TNFAIP3 antibody [EPR2663] (ab92324)

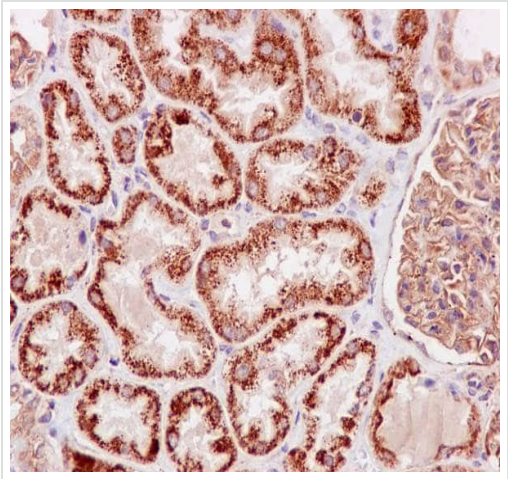
Anti-TNFAIP3 antibody [EPR2663] (ab92324) at 1/3000 dilution (purified) + Jurkat cell lysate - treated with TNF (**ab9642**) and TPA at 10 µg

Secondary

Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 90 kDa
Observed band size: 80 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



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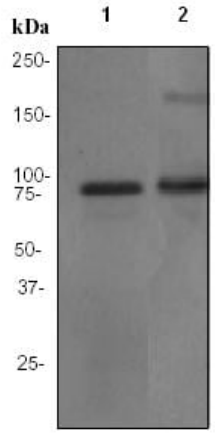
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney tissue labelling TNFAIP3 with purified ab92324 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit IgG was used as the secondary antibody. Counterstained with Hematoxylin.



Western blot - Anti-TNFAIP3 antibody [EPR2663] (ab92324)

All lanes : Anti-TNFAIP3 antibody [EPR2663] (ab92324) at 1/1000 dilution (unpurified)

Lane 1 : Jurkat cells treated with TNF (**ab9642**) and TPA

Lane 2 : Daudi cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : HRP labelled goat anti-rabbit IgG at 1/2000 dilution

Predicted band size: 90 kDa

Anti-TNFAIP3 antibody [EPR2663] (ab92324)

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