abcam

Product datasheet

Anti-TNFAIP3 antibody [EPR2663] - BSA and Azide free ab227987

KO VALIDATED Recombinant RabMAb

9 References 6 Images

Overview

Product name	Anti-TNFAIP3 antibody [EPR2663] - BSA and Azide free
Description	Rabbit monoclonal [EPR2663] to TNFAIP3 - BSA and Azide free
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	ab227987 is the carrier-free version of **ab92324**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR2663
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab227987 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

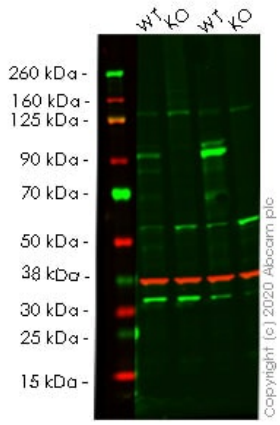
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 90 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. **Mouse species is recommended 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] - BSA and Azide free (ab227987)

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.

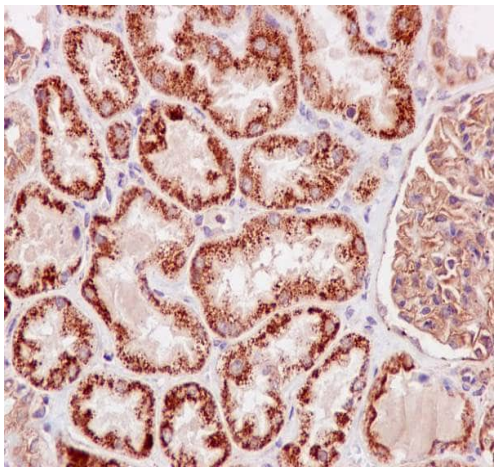
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (**ab92324**).

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.

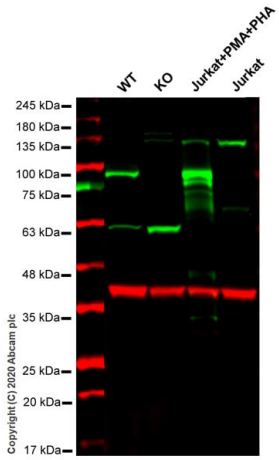
ab92324 was shown to react with TNFAIP3 in wild-type A549 cells in western blot. Loss of signal was observed when knockout cell line **ab266946** (knockout cell lysate **ab257114**) was used. Wild-type A549 and TNFAIP3 knockout A549 cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 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] - BSA and Azide free (ab227987)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92324**).



Western blot - Anti-TNFAIP3 antibody [EPR2663] - BSA and Azide free (ab227987)

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.

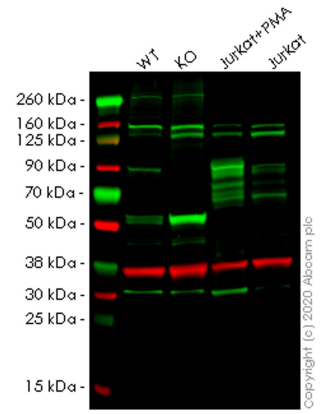
Predicted band size: 90 kDa
Observed band size: 80 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab92324**).

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] - BSA and Azide free (ab227987)

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

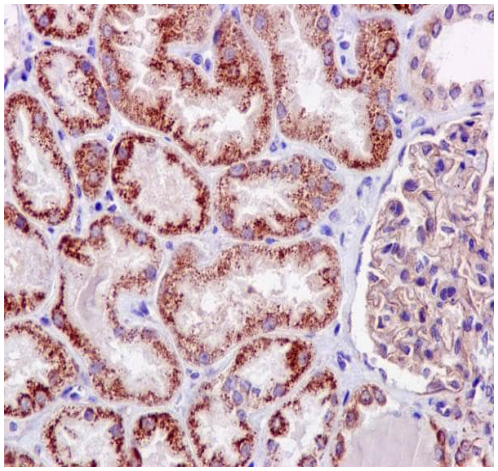
Predicted band size: 90 kDa
Observed band size: 80 kDa

This data was developed using **ab92324**, the same antibody clone in a different buffer formulation.

Lanes 1-4: Merged signal (red and green). Green - **ab92324**

observed at 80 kDa. Red - loading control **ab8245** observed at 36 kDa.

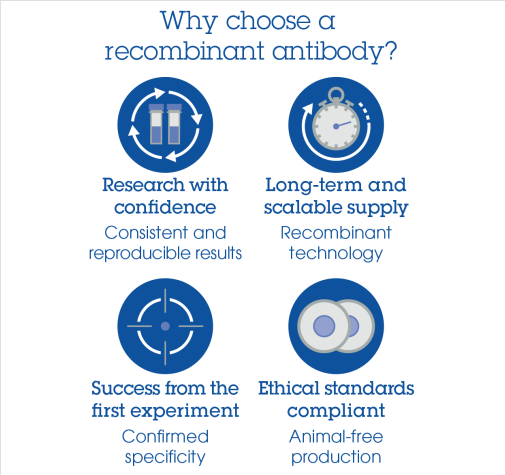
ab92324 Anti-TNFAIP3 antibody [EPR2663] was shown to specifically react with TNFAIP3 in wild-type A549 cells. Loss of signal was observed when knockout cell line **ab266945** (knockout cell lysate **ab257113**) was used. Wild-type and TNFAIP3 knockout samples were subjected to SDS-PAGE. **ab92324** and 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TNFAIP3 antibody [EPR2663] - BSA and Azide free (ab227987)

This IHC data was generated using the same anti-TNFAIP3 antibody clone, EPR2663, in a different buffer formulation (cat# **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.



Anti-TNFAIP3 antibody [EPR2663] - BSA and Azide free (ab227987)

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