abcam

Product datasheet

Anti-TNFAIP3 antibody [59A426] ab13597

KO VALIDATED

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Overview

Product name	Anti-TNFAIP3 antibody [59A426]
Description	Mouse monoclonal [59A426] to TNFAIP3
Host species	Mouse
Tested applications	**Suitable for:** Flow Cyt (Intra), IHC-P, WB **Unsuitable for:** ICC
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant full length protein corresponding to Human TNFAIP3. Database link: **P21580**
Epitope	The epitope has been mapped to the C-terminal portion of A20, amino acids 440-790.
Positive control	WB: Daudi and HeLa cell lysates. Flow Cyt (Intra): HepG2 cells
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS
Purity	Protein G purified
Clonality	Monoclonal
Clone number	59A426

Isotype IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab13597 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)		Use 1-2µg for 10^6 cells. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
IHC-P		Use at an assay dependent concentration.
WB	★★★☆☆ (1)	Use a concentration of 2 - 4 µg/ml. Detects a band of approximately 70 kDa.

Application notes Is unsuitable for ICC.

Target

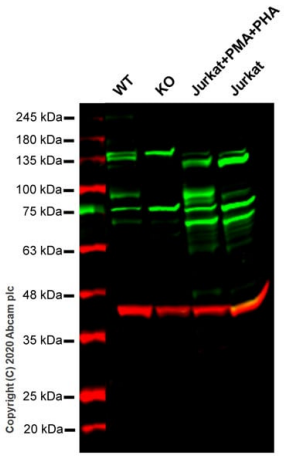
Function Ubiquitin-editing enzyme that contains both ubiquitin ligase and deubiquitinase activities. Essential component of a ubiquitin-editing protein complex, comprising also RNF11, ITCH and TAX1BP1, that ensures the transient nature of inflammatory signaling pathways. Upon TNF stimulation, deubiquitinates '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 [59A426] (ab13597)

All lanes : Anti-TNFAIP3 antibody [59A426] (ab13597) at 1/500 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

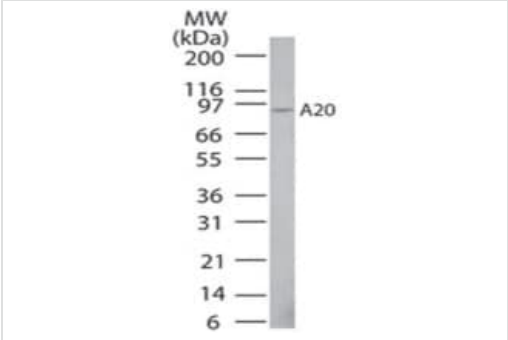
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 80 kDa

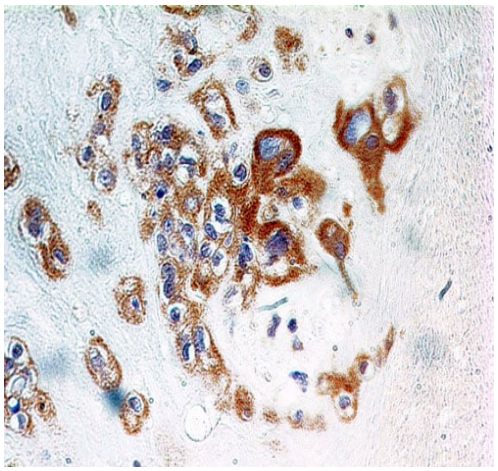
Lanes 1-4: Merged signal (red and green). Green - ab13597 observed at 80 kDa. Red - loading control, **ab181602** observed at 37 kDa.

ab13597 Anti-TNFAIP3 antibody [59A426] 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. ab13597 and Anti-GAPDH antibody [EPR16891] - Loading Control (**ab181602**) were incubated overnight at 4°C at 1 in 500 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216777**) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-TNFAIP3 antibody [59A426] (ab13597)

Anti-TNFAIP3 antibody [59A426] (ab13597) at 4 µg/ml + Jurkat cell lysate



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TNFAIP3 antibody [59A426] (ab13597)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human placenta tissue labelling TNFAIP3 with ab13597 at 5µg/ml. Staining was enhanced by boiling tissue sections in 10mM sodium citrate buffer, pH6.0 for 10-20 minutes followed by cooling at room temperature for 20 minutes.

Western blot - Anti-TNFAIP3 antibody [59A426] (ab13597)

All lanes : Anti-TNFAIP3 antibody [59A426] (ab13597) at 1 µg/ml

Lane 1 : Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate
Lane 2 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

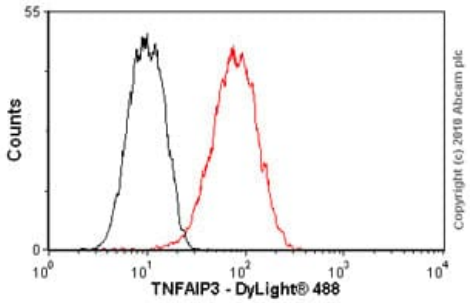
Secondary
All lanes : Goat Anti-Mouse IgG H&L (HRP) preadsorbed (**ab97040**) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Observed band size: 90 kDa
Additional bands at: 15 kDa, 34 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 20 minutes



Flow Cytometry (Intracellular) - Anti-TNFAIP3 antibody [59A426] (ab13597)

Overlay histogram showing HepG2 cells stained with ab13597 (red line). The cells were fixed with methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab13597, 2µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a decreased signal in HepG2 cells fixed with methanol (5 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.

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