Product name: Anti-TNFAIP3 antibody [59A426] ab13597

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.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>Flow Cyt (Intra)</td>
<td></td>
<td>Use 1-2µg for 10^6 cells. Ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.</td>
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<tr>
<td>IHC-P</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>WB</td>
<td>★★★☆☆☆ (1)</td>
<td>Use a concentration of 2 - 4 µg/ml. Detects a band of approximately 70 kDa.</td>
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</tbody>
</table>

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

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.

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

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.

**Please note:** All products are “FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES”

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