

# Anti-TNFAIP3 Antibody [SN07-31]

ET1611-40



<b>Product Type:</b>	Recombinant Rabbit monoclonal IgG, primary antibodies
<b>Species reactivity:</b>	Human
<b>Applications:</b>	WB, IF-Cell, IF-Tissue, IHC-P, FC
<b>Molecular Wt:</b>	Predicted band size: 90 kDa
<b>Clone number:</b>	SN07-31

**Description:** A20 is a Cys2/Cys2 zinc finger protein that is induced by a variety of inflammatory stimuli and regulates gene expression. Specifically, A20 is induced by tumor necrosis factor (TNF) and interleukin 1 (IL-1), and acts as a negative regulator of nuclear factor  $\kappa$  B (NF $\kappa$ B) gene expression. By inhibiting NF $\kappa$ B activation, A20 plays a critical role in terminating NF $\kappa$ B responses to various stimuli. Although the C-terminal region of A20 contains seven zinc finger domains, only four of these domains are required for in vitro inhibition of TNF-induced NF $\kappa$ B activation. A20 also interacts with several other proteins, such as TRAF2, TRAF6 and I $\kappa$ B kinase (IKK)  $\gamma$  protein, and can thereby inhibit cell death. TXBP151, a novel A20-binding protein, may mediate the anti-apoptotic activity of A20. Involved in the negative feedback regulation of signal transduction, A20 and A20-binding proteins may be useful as novel therapeutic tools in the treatment of a variety of diseases.

**Immunogen:** Synthetic peptide within Human TNFAIP3 aa 491-540 / 790.

**Positive control:** Jurkat cell lysates, Hela, A549, HepG2, human kidney tissue.

**Subcellular location:** Cytoplasm, Nucleus, Lysosome.

**Database links:** SwissProt: P21580 Human

**Recommended Dilutions:**

<b>WB</b>	1:500-1:1,000
<b>IF-Cell</b>	1:100-1:500
<b>IF-Tissue</b>	1:100-1:500
<b>IHC-P</b>	1:50-1:200
<b>FC</b>	1:50-1:100

**Storage Buffer:** 1\*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

**Storage Instruction:** Shipped at 4°C. Store at +4°C short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20°C long term.

**Purity:** Protein A affinity purified.

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Orders:0086-571-88062880

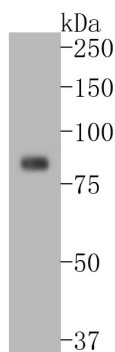
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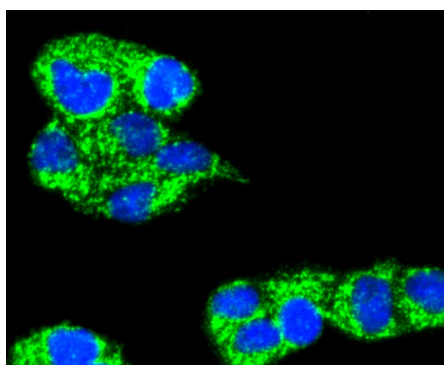
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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

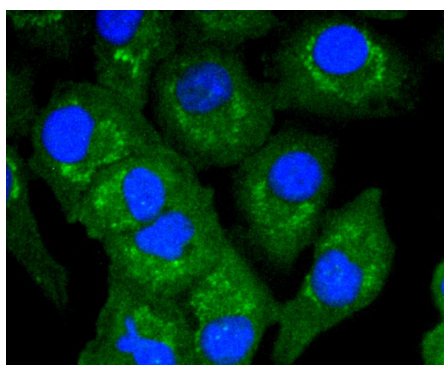
## Images



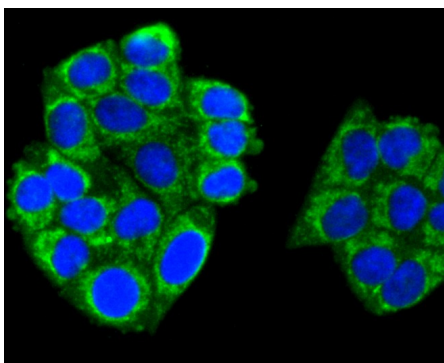
**Fig1:** Western blot analysis of TNFAIP3 on Jurkat cell lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1611-40, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.



**Fig2:** ICC staining of TNFAIP3 in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1611-40, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).



**Fig3:** ICC staining of TNFAIP3 in A549 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1611-40, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).



**Fig4:** ICC staining of TNFAIP3 in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1611-40, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

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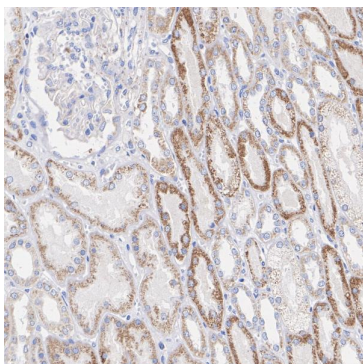
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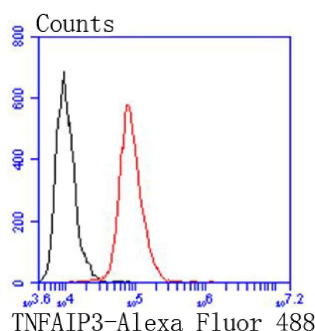
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**Fig5:** Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-TNFAIP3 antibody (ET1611-40) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1611-40) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig6:** Flow cytometric analysis of TNFAIP3 was done on HepG2 cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1611-40, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

**Note:** All products are “FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE”.

### Background References

1. Altonsy MO et al. Context-dependent cooperation between nuclear factor B (NF-B) and the glucocorticoid receptor at a TNFAIP3 intronic enhancer: a mechanism to maintain negative feedback control of inflammation. *J Biol Chem* 289:8231-9 (2014).
2. Haemmig S et al. miR-125b controls apoptosis and temozolomide resistance by targeting TNFAIP3 and NKIRAS2 in glioblastomas. *Cell Death Dis* 5:e1279 (2014).

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