Product Datasheet

A20/TNFAIP3 Antibody (59A426) - BSA Free
NBP1-77533

Unit Size: 0.1 mg

Store at -20C. Avoid freeze-thaw cycles.

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Publications: 42

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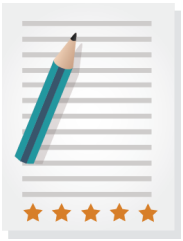
Updated 9/9/2025 v.20.1



NBP1-77533

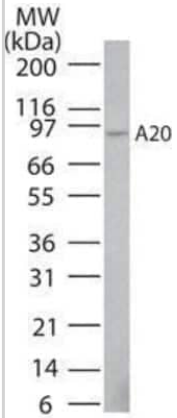
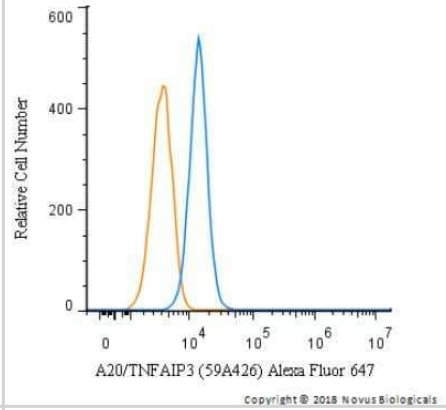
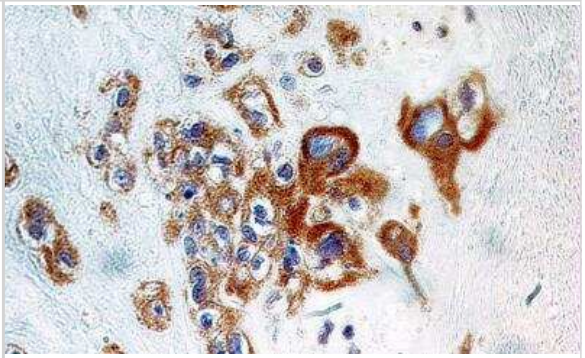
A20/TNFAIP3 Antibody (59A426) - BSA Free

Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	59A426
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS

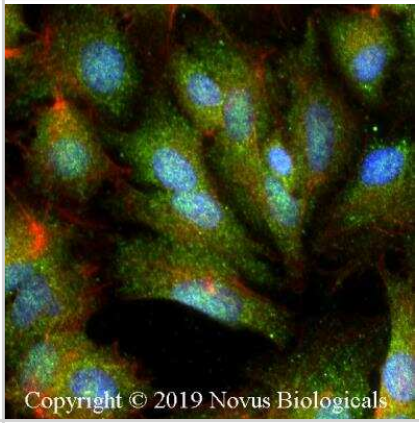
Product Description	
Description	Novus Biologicals Mouse A20/TNFAIP3 Antibody (59A426) - BSA Free (NBP1-77533) is a monoclonal antibody validated for use in IHC, WB, Flow, ICC/IF, Simple Western and IP. Anti-A20/TNFAIP3 Antibody: Cited in 42 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Mouse
Gene ID	7128
Gene Symbol	TNFAIP3
Species	Human, Mouse, Rat
Immunogen	Full length recombinant human A20/TNFAIP3 Antibody (59A426). The epitope has been mapped to the C-terminal portion of A20 (amino acids 440-790)

Product Application Details	
Applications	Western Blot, Simple Western, Immunohistochemistry-Paraffin, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunoprecipitation, CyTOF-ready
Recommended Dilutions	Western Blot 2-4 ug/ml, Simple Western 1:100, Flow Cytometry 2.5 ug/ml, Immunohistochemistry 5 - 10 ug/ml, Immunocytochemistry/ Immunofluorescence 10 ug/ml, Immunoprecipitation 1-2 ug/ml, Immunohistochemistry-Paraffin 5 - 10 ug/ml. Use reported in scientific literature (Metellus (2010)), Flow (Intracellular) 2.5 ug/ml. Use reported in scientific literature (Hjelmeland (2010)), CyTOF-ready
Application Notes	Multiple A20 cleavage fragments have been described in Western Blot, see Coornaert (2008) and Hailfinger (2009) for additional details. In Simple Western only 10 - 15 ul of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in Jurkat lysate 0.05 mg/mL, separated by Size, antibody dilution of 1:100, apparent MW was 102 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue. This antibody is CyTOF ready.

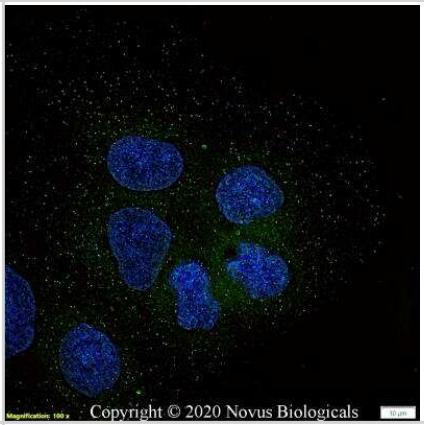
Images

Western Blot: A20/TNFAIP3 Antibody (59A426) [NBP1-77533] - Human Jurkat lysate probed with A20 antibody at 4 ug/ml.	
Flow Cytometry: A20/TNFAIP3 Antibody (59A426) [NBP1-77533] - An intracellular stain was performed on SK-MEL-28 cells with A20/TNFAIP3 (59A426) antibody NBP1-77533AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.	
Simple Western: A20/TNFAIP3 Antibody (59A426) [NBP1-77533] - Lane view shows a specific band for A20/TNFAIP3 in 0.05 mg/ml of Jurkat lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Immunohistochemistry-Paraffin: A20/TNFAIP3 Antibody (59A426) [NBP1-77533] - Human placenta probed with A20 antibody at 5 ug/ml, cytoplasmic staining of decidual cells is observed. Human tissue TMA was used for this test. Staining of formalin-fixed tissues is enhanced by boiling tissue sections in 10 mM sodium citrate buffer, pH 6.0 for 10-20 min followed by cooling at RT for 20 min.	

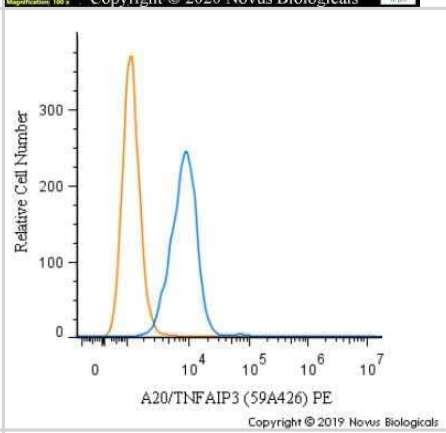
Immunocytochemistry/Immunofluorescence: A20/TNFAIP3 Antibody (59A426) [NBP1-77533] - HepG2 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton-X100. The cells were incubated with anti-A20/TNFAIP3 (59A426) at 10 ug/ml overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:500 dilution. Actin was detected with Phalloidin 568 (Red) at a 1:200 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective



Immunocytochemistry/Immunofluorescence: A20/TNFAIP3 Antibody (59A426) [NBP1-77533] - Caco-2 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-A20/TNFAIP3 Antibody [59A426] NBP1-77533 at 2 ug/ml overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



Flow Cytometry: A20/TNFAIP3 Antibody (59A426) [NBP1-77533] - An intracellular stain was performed on RH-30 cells with A20/TNFAIP3 Antibody (59A426) NBP1-77533PE (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Phycoerythrin.



A20/TNFAIP3 (59A426) was detected in immersion fixed Caco-2 human colorectal adenocarcinoma cell line using Mouse anti-A20/TNFAIP3 (59A426) Protein G Purified Monoclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NBP1-77533AF647) (light blue) at 10 µg/mL overnight at 4C. Cells were counterstained with DAPI (dark blue). Cells were imaged using a 40X objective.

Publications

Wang yT, Liu Ty, Shen CH et al. K48/K63-linked polyubiquitination of ATG9A by TRAF6 E3 ligase regulates oxidative stress-induced autophagy Cell reports 2022-02-22 [PMID: 35196483] (WB, ICC/IF, Mouse, Human)

Tran VT, Ju H Fluorescence Based on Surface Plasmon Coupled Emission for Ultrahigh Sensitivity Immunoassay of Cardiac Troponin I Biomedicines 2021-04-21 [PMID: 33919217]

Feoktistova M, Makarov R, Brenji S et al. A20 Promotes Ripoptosome Formation and TNF-Induced Apoptosis via cIAPs Regulation and NIK Stabilization in Keratinocytes Cells 2020-02-03 [PMID: 32028675] (WB, Human)

Ma J, Xiao Y, Tian B et al. Genome-wide analyses of long non-coding RNA expression profiles and functional network analysis in esophageal squamous cell carcinoma Sci Rep 2019-06-24 [PMID: 31235759] (WB, Human)

Lim MCC, Maubach G, Sokolova O et al. Pathogen-induced ubiquitin-editing enzyme A20 bifunctionally shuts off NF-kB and caspase-8-dependent apoptotic cell death. Cell Death Differ. 2017-06-02 [PMID: 28574503] (WB, Human)

Reihill JA, Malcomson B, Bertelsen A et al. Induction of the inflammatory regulator A20 by gibberellic acid in airway epithelial cells. Br. J. Pharmacol. 2015-05-25 [PMID: 26013851] (WB, Human)

Lai Ting-Yu, Wu Shang-Duen, Tsai Mong-Hsun et al. Transcription of Tnfaip3 is regulated by NF-kappaB and p38 via C/EBPbeta in activated macrophages. PLoS One. 2013-01-01 [PMID: 24023826] (Mouse)

Xuan NT, Wang X, Nishanth G et al. A20 expression in dendritic cells protects mice from LPS-induced mortality. Eur. J. Immunol. 2014-12-04 [PMID: 25530110] (WB, Mouse)

Details:
A20 antibody used for WB on lysates of bone marrow-derived dendritic cells (BMDCs) isolated from CD11c-Cre A20fl/fl and A20fl/fl control mice (Figure 1A).

De A, Dainichi T, Rathinam CV, Ghosh S. The deubiquitinase activity of A20 is dispensable for NF-kB signaling. EMBO Rep. 2014-05-30 [PMID: 24878851] (WB, Mouse)

Details:
Primary mouse bone marrow derived macrophages, WB: Figs 1C, 5B, 5D; IP: S9. For IP, the immunoprecipitated A20 was was used in an in vitro deubiquitinase (DUB) assay (S9). Notes: TNFa and LPS treatment upregulated A20 expression (Fig 1C). The A20 mAb reco

Frenzel LP, Claus R, Plume N et al. Sustained NF-kappaB activity in chronic lymphocytic leukemia is independent of genetic and epigenetic alterations in the TNFAIP3 (A20) locus. Int J Cancer. 2011-05-15 [PMID: 20669229]

Details:
WB: B cells isolated from healthy blood donors and from patients with CLL, Fig 3.

Salaun B, Lebecque S, Matikainen S et al. Toll-like receptor 3 expressed by melanoma cells as a target for therapy? Clin Cancer Res. 2007-08-01 [PMID: 17671143]

Details:
WB (Fig 1): Me 260 and Me 300 human melanoma cell lines treated with IFNa, Poly(I:C) or IFNa + Poly(I;C). Cells had a low or undetectable basal level of A20. A20 expression was upregulated in Me 260 with treatment, most notably with IFNa followed by Poly(I:C).

Metellus P, Coulibaly B, Nanni I et al. Prognostic impact of O6-methylguanine-DNA methyltransferase silencing in patients with recurrent glioblastoma multiforme who undergo surgery and carmustine wafer implantation: a prospective patient cohort. Cancer. 2009-10-15 [PMID: 19637364]

Details:
IHC(P): human glioblastoma multiforme, results described but not shown.

More publications at http://www.novusbio.com/NBP1-77533

Procedures

Western Blot Protocol for A20/TNFAIP3 Antibody (NBP1-77533)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers inst

Flow (Intracellular) Protocol for A20/TNFAIP3 Antibody (NBP1-77533)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 uL for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.

a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.

4. Re-suspend cells to a concentration of 1 x 106 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 uL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeablization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

1. Fix the cells by adding 100 uL fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 uL of a permeabization buffer to every 1 x 106 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.

a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.

b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).

3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 uL of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 ug per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.

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Products Related to NBP1-77533

NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB7539	Goat anti-Mouse IgG (H+L) Secondary Antibody [HRP]
NBP1-43319-0.5mg	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1)

Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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