

#4625 Store at -20°C

A20/TNFAIP3 Antibody



100 µl
 (10 Western mini-blots)

Orders ■ 877-616-CELL (2355)
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New 02/08

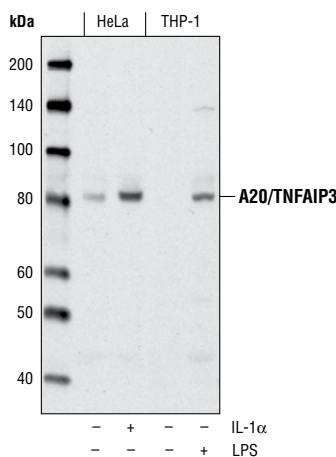
This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP Endogenous	H, (Mk)	82 kDa	Rabbit

Background: A20, also referred to as TNF- α -induced protein 3 (TNFAIP3), is cytokine inducible protein which functions to inhibit apoptosis and activate NF- κ B (1,2). It was first identified as a TNF- α inducible primary response gene in human umbilical vein endothelial cells, and encodes a 790-amino acid protein containing seven Cys2/Cys2-zinc finger motifs (3). Constitutive expression of A20 is observed in lymphoid tissues (4), but it is transiently expressed in a variety of cell types in response to inflammatory signals such as TNF- α (3,5), IL-1 (3,5), phorbol esters (6), and LPS (7). Expression of A20 can confer resistance to apoptosis and NF- κ B activation triggered by these signals, probably through interference with TRAF (TNF receptor associated factor) family members (8,9), as well as interaction with the NF- κ B inhibiting protein ABIN (10). Studies also show that it contains site-specific ubiquitin modifying activity that can contribute to its biological functions (11,12). The amino-terminal of A20 contains de-ubiquitinating (DUB) activity for Lys63 branches, such as those found in TRAF6 and RIP, while the carboxyl-terminus contains ubiquitin ligase (E3) activity for Lys48 branches of the same substrates leading to their degradation (12).

Specificity/Sensitivity: A20/TNFAIP3 Antibody detects endogenous levels of total A20/TNFAIP3 protein.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to residues surrounding Gly267 of human A20/TNFAIP3. Antibodies were purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from HeLa cells, untreated or treated with IL-1 α , and from differentiated THP-1 cells, untreated or treated with LPS, using A20/TNFAIP3 Antibody.

Background References:

- (1) Beyaert, R. et al. (2000) *Biochem Pharmacol* 60, 1143–51.
- (2) Lee, E.G. et al. (2000) *Science* 289, 2350–4.
- (3) Dixit, V.M. et al. (1990) *J Biol Chem* 265, 2973–8.
- (4) Tewari, M. et al. (1995) *J Immunol* 154, 1699–706.
- (5) Jäättelä, M. et al. (1996) *J Immunol* 156, 1166–73.
- (6) Laherty, C.D. et al. (1993) *J Biol Chem* 268, 5032–9.
- (7) Hu, X. et al. (1998) *Blood* 92, 2759–65.
- (8) Song, H.Y. et al. (1996) *Proc Natl Acad Sci USA* 93, 6721–5.
- (9) Heyninck, K. and Beyaert, R. (1999) *FEBS Lett* 442, 147–50.
- (10) Heyninck, K. et al. (1999) *J Cell Biol* 145, 1471–82.
- (11) Evans, P.C. et al. (2004) *Biochem J* 378, 727–34.
- (12) Lin, S.C. et al. (2008) *J Mol Biol* 376, 526–40.

Entrez-Gene ID # 7128
Swiss-Prot Acc. # P21580

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. *Do not aliquot the antibody.*

***Species cross-reactivity is determined by Western blot.**

Recommended Antibody Dilutions:

Western Blotting 1:1000
 Immunoprecipitation 1:25

Companion Products:

- NF- κ B p65 (C22B4) Rabbit mAb #4764
- RIP Antibody #4926
- TNF-R1 (C25C1) Rabbit mAb #3736
- TNF-R2 Antibody #3727
- TRADD (7G8) Rabbit mAb #3684
- TRAF1 (45D3) Rabbit mAb #4715
- TRAF2 Antibody (Human Specific) #4724
- TRAF3 Antibody #4729
- Phototope[®]-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
- Anti-rabbit IgG, HRP-linked Antibody #7074
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder #7727
- 20X LumiGLO[®] Reagent and 20X Peroxide #7003

IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus
 Species enclosed in parentheses are predicted to react based on 100% sequence homology.

F—Flow cytometry E—ELISA D—DELFI[®]
 Z—zebra fish B—bovine All—all species expected

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
10. **Phototope[®]-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO[®] chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO[®] incubation and declines over the following 2 hours.

Immunoprecipitation Protocol / (For Analysis By Western Immunoblotting)

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X Cell Lysis Buffer:** 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM Sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM Na_3VO_4 , 1 $\mu\text{g/ml}$ Leupeptin

NOTE: Add 1 mM PMSF immediately prior to use.

1. **Transfer Buffer:** 25 mM Tris base, 0.2 mM glycine, 20% methanol (pH 8.5)
2. **Protein A or G Agarose Beads:** (Can be stored for 2 weeks at 4°C.) Please prepare according to manufacturer's instructions. Use Protein A for rabbit IgG pull down and Protein G for mouse IgG pull down.
3. **3X SDS Sample Buffer:** 187.5 mM Tris-HCl (pH 6.8 at 25°C), 6% w/v SDS, 30% glycerol, 150 mM DTT, 0.03% w/v bromophenol blue

B Preparing Cell Lysates

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X cell lysis buffer to each plate (10 cm) and incubate the plates on ice for 5 minutes.
4. Scrape cells off the plates and transfer to microcentrifuge tubes. Keep on ice.
5. Sonicate samples on ice three times for 5 seconds each.
6. Microcentrifuge for 10 minutes at 14,000 X g, 4°C, and transfer the supernatant to a new tube. If necessary, lysate can be stored at -80°C.

C Immunoprecipitation

Optional: It may be necessary to perform a lysate pre-clearing step to reduce non-specific binding to the Protein A/G agarose beads (See section below).

1. Take 200 μl cell lysate and add primary antibody. Incubate with gentle rocking overnight at 4°C.
2. Add either protein A or G agarose beads (20 μl of 50% bead slurry). Incubate with gentle rocking for 1–3 hours at 4°C.
3. Microcentrifuge for 30 seconds at 4°C. Wash pellet five times with 500 μl of 1X cell lysis buffer. Keep on ice during washes.
4. Resuspend the pellet with 20 μl 3X SDS sample buffer. Vortex, then microcentrifuge for 30 seconds.
5. Heat the sample to 95–100°C for 2–5 minutes and microcentrifuge for 1 minute at 14,000 X g.
6. Load the sample (15–30 μl) on SDS-PAGE gel (12–15%).
7. Analyze sample by Western blotting (see Western Immunoblotting Protocol).

Cell Lysate Pre-Clearing (Optional)

1. Take 200 μl cell lysate and add to either Protein A or G agarose beads (20 μl of 50% bead slurry).
2. Incubate at 4°C for 30 – 60 minutes.
3. Spin for 10 minutes at 4°C. Transfer the supernatant to a fresh tube.
4. Proceed to step 1 of Immunoprecipitation.