**For Research Use Only.
Not for use in diagnostic procedures.**

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MONOCLONAL ANTIBODY

Anti-TRAF2 mAb

Code No.	Clone	Subclass	Quantity	Concentration
M112-3	6F8	Rat IgG2a	100 µL	1 mg/mL

BACKGROUND: TNF receptor associated factor 2 (TRAF2) is thought to be a common signal transducer that associates with the cytoplasmic domains of the tumor necrosis factor receptor (TNFR) superfamily such as TNFR2, CD40 and CD30. TRAF2 indirectly associates with TNFR1 through the association of TRADD. These associations result in the activation of NF-κB. TRAF2 was isolated as the TNFR2 associated protein by biochemical method. It contains an N-terminal RING finger motif and a C-terminal TRAF domain. Moreover TRAF2 interacts with cIAP1/HIAP2, cIAP2/HIAP1, I-TRAF/TANK, TRAF1, RIP and A20.

SOURCE: This antibody was purified from hybridoma (clone 6F8) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Wister rat splenocyte immunized with the recombinant full-length mouse TRAF2.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with human, mouse and rat TRAF2 on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; 5 µg/350 µL of cell extract from 5 x 10^6 cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested*
*It is reported that clone 6F8 can be used in this application in the reference number 1)

Detailed procedure is provided in the following **PROTOCOLS**.

INTENDED USE:

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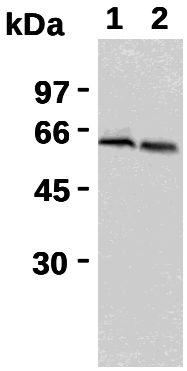
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Raji, HeLa	NIH/3T3	PC12
Reactivity on WB	+	+	+

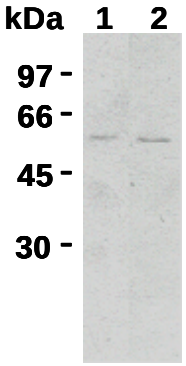
REFERENCES:

1) Iwata, S., *et al., J Allergy Clin Immunol.* **129**, 1594-601 (2012) [FCM]
2) Cheung, T. C., *et al., PNAS*, **106**, 6244-6249 (2009)
3) Mauro, C., *et al., J. Biol. Chem.* **281**, 2631-2638 (2006)
4) Kim, W. J., *et al., Mol. Cell Biol.* **25**, 2450-2462 (2005)

Clone 6F8 is used in the reference number 1).



Western blot analysis of human TRAF2 expression in Raji cells (1) and HeLa cells (2) using M112-3.



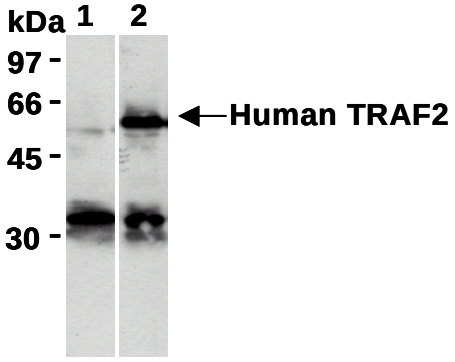
Western blot analysis of mouse TRAF2 expression in PC12 cells (1) and NIH/3T3 cells (2) using M112-3.

PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 1 mL of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 20 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% Methanol). See the manufacturer's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
5) Incubate the membrane for 1 hour at room temperature with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS**. (The concentration of antibody will depend on the conditions.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
7) Incubate the membrane with 1:10,000 HRP-conjugated anti-rat IgG (MBL; code no. IM-0825) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS-T (5 minutes x 3 times).
9) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
10) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
11) Expose the membrane onto an X-ray film in a dark room for 3 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive controls for Western blotting; Raji, HeLa, NIH/3T3, PC12)



Immunoprecipitation of human TRAF2 from HeLa cells with Rat IgG2a (1) or M112-3 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with M112-3.*

Immunoprecipitation

1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 1 mL of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing protease inhibitors at appropriate concentrations. Incubate it at 4°C with rotating for 30 minutes; thereafter, briefly sonicate the mixture (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another fresh tube.
3) Add primary antibody as suggested in the **APPLICATIONS** into 300 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
4) Add 20 µL of 50% protein G agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
5) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
6) Resuspend the beads with cold Lysis buffer.
7) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
8) Repeat steps 6)-7) 2-4 times.
9) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis.

(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; HeLa)

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