

PN IM3280 Monoclonal Antibody **TRAF2**

Form	Unconjugated	Clone	Polyclonal
Quantity	100µg	Isotype	N/A
Presentation	Freeze-dried	Species	Rabbit
Purity	Purified Igs		

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

SPECIFICITY

The tumor necrosis factor receptor (TNFR)-associated factor 2 (TRAF2) is thought to be a common signal transducer that associates with the cytoplasmic domains of members of the TNFR superfamily, such as TNFR2 (1), CD40 (2) and CD30 (3, 4); TRAF2 is indirectly in relationship with TNFR1 through the association of TRADD (5, 6). These connections result in the activation of NF-κB. TRAF2 was isolated as a TNFR2-associated protein by biochemical method. It contains an N-terminal RING finger motif, 5 zinc finger motifs and a C-terminal TRAF domain (1). Moreover, TRAF2 interacts with cIAP1,2 (7), I-TRAF / TANK (8, 9), TRAF1 (1), RIP (10) and A20 (11).

The present polyclonal antibody reacts with mouse and human TRAF2 (56 kDa and 58 kDa, respectively) in Western blotting, using total cell lysate from murine NIH3T3, WR19L12a, and human ZR75-1, Raji cell line, or HA-tag-TRAF2.

Species cross reactivity: Mouse and Human.

Positive control: HA-tag-TRAF2 expressed cell lysate (code: 592-P, lyophilized form). The HA-tag-TRAF2 overexpressed cell lysate was derived from pCMV-HA-tag-TRAF2 transfected 293T cell line.

APPLICATION

Immunoblotting

BUFFER

This antibody is provided in phosphate-buffered saline (PBS), containing 1% sucrose.

STORAGE CONDITIONS AND STABILITY

This freeze-dried form may be stored at 2-8°C until the expiration date stated on the vial label.

No preservative has been added.

REAGENT PREPARATION

This antibody is provided in freeze-dried presentation and must be reconstituted with 100 µL of distilled water. Bring reagent to 18-25°C prior to use.

PROCEDURE

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) & Immunoblotting

Use a working dilution of 1:1000 for chemiluminescence detection.

1. Boil all samples for 3-5 minutes. To each well of an SDS-PAGE, load 10 µL of cell lysate or tissue homogenate (5-20 µg total protein) and perform electrophoresis in a 1 mm thick gel.
2. Transfer to polyvinylidene difluoride (PVDF) membrane at 10 V for 1 hour in a semi-dry transfer system. (transfer buffer: 25 mM Tris, 190 mM glycine, 20% MeOH).
3. The transferred proteins can be visualized by staining the membrane for 1 minute with 0.1% Ponceau S (SIGMA Cat. No. P 7170) in 5% acetic acid. Rinse the membrane with PBS.
4. Non-specific binding sites are blocked by immersing the membrane in 5% skim milk / PBS / 0.05% Tween 20 for 1 hour at room temperature (18-25°C), or overnight at 4°C.
5. Incubate with primary antibody at the recommended dilution (the concentration of antibody to be used may vary depending on the studied tissue) for 1 hour at room temperature.
6. Wash the membrane 3 times for 5-10 minutes per wash with PBS / 0.05% Tween 20.
7. Incubate with Horseradish Peroxidase-conjugated goat anti-mouse (PN IM0817) diluted in PBS / 0.05% Tween 20 for 45 minutes at room temperature.
8. Wash the membrane 3 times for 10 minutes per wash with PBS / 0.05% Tween 20.
9. Incubate with Amersham ECL Reagent for 1 minute. Drain membrane, remove excess ECL Reagent by dabbing with a Kimwipe, and seal in plastic wrap.
10. Expose to ECL hyperfilm in a dark room for 30 seconds. Develop as usual for autoradiogram or X-ray. The conditions for development and exposure may vary.

STATEMENT OF WARNINGS

1. Specimens, samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.

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2. Never pipet by mouth and avoid contact of samples with skin and mucous membranes
3. Do not use antibody beyond the expiration date on the label.
4. Do not expose reagents to strong light during storage or incubation.
5. Avoid microbial contamination of reagents or incorrect results might occur.

SELECTED RESEARCH REFERENCES

1. [292] Rothe, M., Wong, S.C., Henzel, W.J., Goeddel, D.V., "A novel family of putative signal transducers associated with the cytoplasmic domain of the 75 kDa tumor necrosis factor receptor", 1994, *Cell*, 78, 681-692.
2. [293] Rothe, M., Sarma, V., Dixit, V.M., Goeddel, D.V., "TRAF2-mediated activation of NF-κB by TNF receptor 2 and CD40", 1995, *Science*, 269, 1424-1427.
3. [294] Gedricht, R.W., Gilfillan, M.C., Duckett, C.S., Van Dongen, J.L., Thompson, C.B., "CD30 contains two binding sites with different specificities for members of the tumor necrosis factor receptor-associated factor family of signal transducing proteins", 1996, *J. Biol. Chem.*, 271, 12852-12858.
4. [295] Lee, S.Y., Park, C.G., Choi, Y., "T cell receptor-dependent cell death of T cell hybridomas mediated by the CD30 cytoplasmic domain in association with tumor necrosis factor receptor-associated factors", 1996, *J. Exp. Med.*, 183, 669-674.
5. [296] Hsu, H., Shu, H.B., Pan, M.-G., Goeddel, D.V., "TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways", 1996, *Cell*, 84, 299-308.
6. [297] Baker, S.J., Reddy, E.P., "Transducers of life and death: TNF receptor superfamily and associated proteins", 1995, *Oncogene*, 12, 1-9.
7. [299] Rothe, M., Pan, M.-G., Henzel, W.J., Ayres, T.M., Goeddel, D.V., "The TNFR2-TRAF signaling complex contains two novel proteins related to baculoviral inhibitor of apoptosis proteins", 1995, *Cell*, 83, 1243-1252.
8. [298] Rothe, M., Xiong, J., Shu, H.-B., Williamson, K., Goddard, A., Goeddel, D.V., "I-TRAF is a novel TRAF-interacting protein that regulates TRAF-mediated signal transduction", 1996, *Proc. Natl. Acad. Sci. USA*, 93, 8241-8246.
9. [300] Cheng, G., Baltimore, D., "TANK, a co-inducer with TRAF2 of TNF-and CD40L-mediated NF-κB activation", 1996, *Genes & Development*, 10, 963-973.
10. [301] Takeuchi, M., Rothe, M., Goeddel, D.V., "Anatomy of TRAF2: distinct domains for nuclear factor-κB activation and association with tumor necrosis factor signaling proteins", 1996, *J. Biol. Chem.*, 271, 19935-19942.
11. [302] Song, H.Y., Rothe, M., Goeddel, D.V., "The tumor necrosis factor-inducible zinc finger protein A20 interacts with TRAF1/TRAF2 and inhibits NF-κB activation", 1996, *Proc. Natl. Acad. Sci. USA*, 93, 6721-6725.