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

POLYCLONAL ANTIBODY

Anti-Mouse TRAF6

Code No.	Quantity	Form
597	100 µL	Purified IgG

BACKGROUND: The TRAF-C domain is involved in homotypic and heterotypic aggregation of TRAFs and in interaction of the TNF-receptor superfamily. TRAF6 cDNA has been identified as sequences homologous to the TRAF-C domain of TRAF2 and as binding the cytoplasmic tail of CD40 using the yeast two-hybrid system. TRAF6 has a TRAF domain in its carboxyl terminus and has a RING finger domain, a cluster of zinc fingers and a coiled-coil domain. TRAF6 interacts strongly with itself, and weakly with both TRAF2 and TRAF3. Overexpression of TRAF6 activates NF-κB. TRAF6 is likely to be a component of the signaling cascade that starts at the IL-1β receptor and results in the activation of NF-κB.

SOURCE: This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with the recombinant N-terminal amino acids of mouse TRAF6 (1-305 aa).

FORMULATION: 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 mouse TRAF6 on Western blotting and Immunoprecipitation. This antibody does not cross react with other TRAF families, TRAF1, TRAF2, TRAF3, TRAF4 and TRAF5.

APPLICATIONS:

Western blotting; 1:1,000 for chemiluminescence detection system
Immunoprecipitation; 2-4 µL/100-500 µL of cell extract from $5x10^6$ cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:

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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Not Tested	NIH/3T3, WR19L, Ba/F3	Not Tested
Reactivity on WB		+	

REFERENCES:

1) Cao, Z., *et al.*, *Nature* **383**, 443-446 (1996)
2) Ishida, T., *et al.*, *J. Biol. Chem.* **271**, 28745-28748 (1996)

RELATED PRODUCTS:

M028-3	Anti-TRAF1 (3D4)
M112-3	Anti-TRAF2 (6F8)
M192-3	Anti-TRAF6 (1F8)
592	Anti-TRAF2 (polyclonal)
CM002-1	Anti-TRAF6 (1B1-2, chicken IgY)
K0039-3	Anti-TNF-R1 (H398)
K0039-4	FITC labeled Anti-TNF-R1 (H398)
K0040-3	Anti-TNF-R2 (80M2)
K0040-4	FITC labeled Anti-TNF-R2 (80M2)
K0157-3	Anti-IKKγ/I-κB Kinase γ (DA10-12)
K0159-3	Anti-IKKγ/I-κB Kinase γ (EA2-6)
M044-3	Anti-XIAP/MIHA/ILP-a (2F1)
CY-1168	AKT/PKB Kinase Assay/Inhibitor Screening Kit
CY-1083	c-Src Kinase Assay/Inhibitor Screening Kit
CY-E1083	c-Src Positive Control
CY-1178	IKKα/β Kinase Assay/Inhibitor Screening Kit
CY-E1178-2	IKKβ Positive Control
CY-E1162-3	C-TAK1 Positive Control
CY-E1162-3	C-TAK1 Positive Control

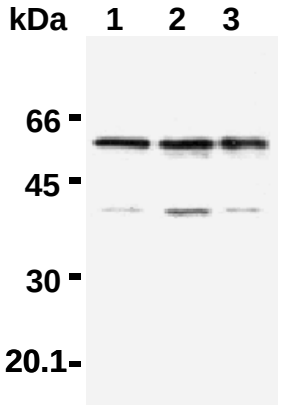
PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (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 tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load

10 μL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) 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% MeOH). See the manufacture's manual for precise transfer procedure.
6) 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^{o}C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 5% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (10 minutes x 3 times).
9) Incubate the membrane with the 1:5,000 HRP-conjugated anti-rabbit IgG (MBL; code no. 458) diluted with 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 5 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; L5178Y, WR19L, NIH/3T3)



Western blot analysis of mouse TRAF6 expression in L5178Y cells (1), WR19L cells (2) and NIH/3T3 cells (3) using 597.

Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4^{o}C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4^{o}C and transfer the supernatant to another tube.
3) Add primary antibody as suggest in the **APPLICATIONS** into 100-500 μL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4^{o}C. Add 20 μL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4^{o}C.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) 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**.)