

# Anti-TIAL1 pAb

**Code No.:** RN059PW

**SOURCE:** rabbit polyclonal antibody, affinity purified

**QUANTITY:** 100 µL

**FORMULATION:** 1 mg/ml in 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

## APPLICATIONS:

Western Blotting 1:1,000

Immunoprecipitation 5 µL/500 µL of cell extract from  $1.5 \times 10^7$  cells

Immunocytochemistry Reported. For more information, please visit our web site <https://ruo.mbl.co.jp/>.

## SPECIES CROSS REACTIVITY on WB:

Species	Human	Mouse	Rat	Hamster
Cells	293T, HeLa, K562	NIH/3T3, WR19L	Rat1	CHO
Reactivity	+	-	+	+

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

**Entrez Gene ID:** 7073 (Human)

## RELATED PRODUCTS

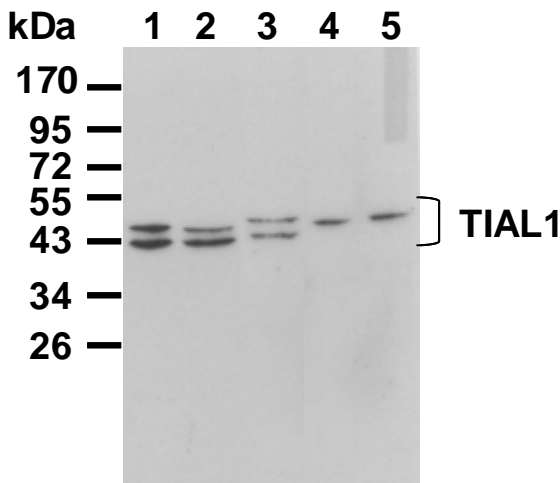
For more information, please visit our web site <https://ruo.mbl.co.jp/>.

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

**SDS-PAGE & Western blotting**

- 1) Wash  $1 \times 10^7$  cells 3 times with PBS and suspend them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 20 seconds).
- 2) Boil the samples for 2 minutes and centrifuge. Load 10  $\mu$ L of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
- 3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> 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.
- 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 with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (10 minutes x 3 times).
- 7) Incubate the membrane with the 1:5,000 HRP-conjugated anti-rabbit IgG (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 8) Wash the membrane with PBS-T (10 minutes x 3 times).
- 9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 10) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; 293T, HeLa, K562, Rat1, CHO)

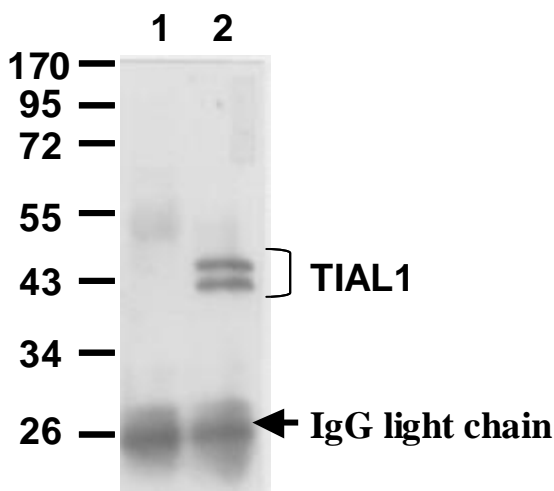


***Western blot analysis of TIAL1 in 293T (1), HeLa (2), K562 (3), Rat1 (4) and CHO (5) using RN059PW.***

**Immunoprecipitation**

- 1) Wash  $3 \times 10^7$  cells 2 times with PBS and resuspend them with 1 mL of ice-cold Lysis buffer (150 mM NaCl, 20 mM Tris-HCl, pH 8.0, 0.1% NP-40, 10 mM EDTA) containing appropriate protease inhibitors. Vortex for 10 seconds, then leave on ice for 10 minutes.
- 2) Centrifuge the tube at 12,000 x g for 5 minutes at 4°C and transfer the supernatant to another tube.
- 3) Add 40 µL of 50% protein A agarose beads slurry resuspended in Lysis Buffer into the supernatant. Incubate it at 4°C with rotating for 1 hour.
- 4) Centrifuge the tube at 2,000 x g for 1 minute at 4°C and transfer the supernatant to another tube (precleared sample).
- 5) Mix both 20 µL of 50% protein A agarose beads slurry resuspended in PBS and normal rabbit IgG (RIP-Assay Kit) or anti-TIAL1 antibody at the amount of suggested in the **APPLICATIONS**, and then add 1 mL of Wash Buffer into each tube. Incubate with gently agitation for 1 hour at 4°C.
- 6) Wash the beads once with ice-cold Lysis Buffer (centrifuge the tube at 2,000 x g for 1 minute). Carefully discard the supernatant using a pipettor without disturbing the beads.
- 7) Add 500 µL of cell lysate (precleared sample of step 4), then incubate with gentle agitation for 3 hours at 4°C.
- 8) Wash the beads 4 times with Wash Buffer (centrifuge the tube at 2,000 x g for 1 minute).
- 9) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 20 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
- 10) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> 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.
- 11) 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.
- 12) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
- 13) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (10 minutes x 3 times).
- 14) Incubate the membrane with the 1:1,000 Rabbit True Blot HRP conjugated anti-Rabbit IgG (eBioscience; code no. 18-8816-33) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 15) Wash the membrane with PBS-T (10 minutes x 3 times).
- 16) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 17) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; HeLa)



**Immunoprecipitation of TIAL1 from HeLa with normal rabbit IgG (1) or RN059PW (2).**

*After immunoprecipitated with antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with RN059PW.*