

## Product datasheet for AM26584AF-N

### TRAF1 / EBI6 (1-410) Mouse Monoclonal Antibody [Clone ID: 3D4]

#### Product data:

Product Type:	Primary Antibodies
Clone Name:	3D4
Applications:	WB
Recommended Dilution:	<b>Western blot:</b> 1 µg/ml for chemiluminescence detection system.
Reactivity:	Mouse
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Recombinant full-length mouse TRAF1 (1-410 aa)
Specificity:	This antibody reacts with mouse TRAF1 (56 kDa) on Western blotting. This antibody doesn't react with mouse TRAF2, TRAF3, TRAF4, TRAF5, and TRAF6.
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservative is contained.
	State: Azide Free
	State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Upon receipt, store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	TNF receptor-associated factor 1
Database Link:	<a href="#">Entrez Gene 22029 Mouse</a> <a href="#">P39428</a>



**Background:** Tumor necrosis factor receptor (TNF-R) superfamily such as TNFR1, TNFR2, CD30 and CD40-mediated signaling is provided by the TRAF (TNF receptor associated factors) family. The members of the TRAF family form either homodimers or heterodimers result in extensive diversity and specificity in their signal transduction pathways. TRAF1 and TRAF2 were found to associate with the cytoplasmic domains of the TNFR2. However, TRAF1 is indirectly associated with TNFR2 via dimerization with TRAF2. TRAF1 and TRAF2 bind independently to the same region of CD30. Both TRAF1 and TRAF2 interact strongly with A20, a TRAF2-mediated NF- $\kappa$ B activation indirectly through the formation of a TRAF2-TRAF1-A20 complex.

**Synonyms:** TNF receptor-associated factor 1

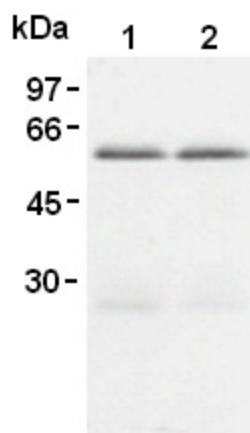
## Note:

This product was originally produced by MBL International.

## Protocol:

## SDS-PAGE &amp; 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<sup>2</sup> for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
- 6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4 °C.
- 7) 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 condition.)
- 8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG diluted with 1% 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 3 minutes.
- 14) Develop the film as usual. The condition for exposure and development may vary.  
(Positive controls for Western blotting; mouse kidney, mouse liver)

**Product images:**

Western blot analysis of Mouse TRAF1 in mouse kidney (1) and mouse liver (2) using AM26584AF-N.