

Anti-Atg14 (Human) mAb

CODE No.	M184-3
CLONALITY	Monoclonal
CLONE	4H8
ISOTYPE	Mouse IgG2 α k
QUANTITY	100 μ L, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	Human Atg14, 167-404 aa (recombinant)
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-CONFIRMED

<u>Western blotting</u>	1 μ g/mL
<u>Immunoprecipitation</u>	2 μ g/300 μ L of cell extract from 3 x 10 ⁶ cells

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cells	HeLa, 293T, A549, Jurkat	NIH/3T3, MEF	PC12	Not tested
Reactivity	+	-	-	

Entrez Gene ID 22863 (Human)

- REFERENCES**
- 1) Zhong, Y., *et al.*, *Nat. Cell Biol.* **11**, 468 (2009)
 - 2) Matsunaga, K., *et al.*, *Nat. Cell Biol.* **11**, 385 (2009)
 - 3) Itakura, E., *et al.*, *Mol. Biol. Cell* **19**, 5360 (2008)

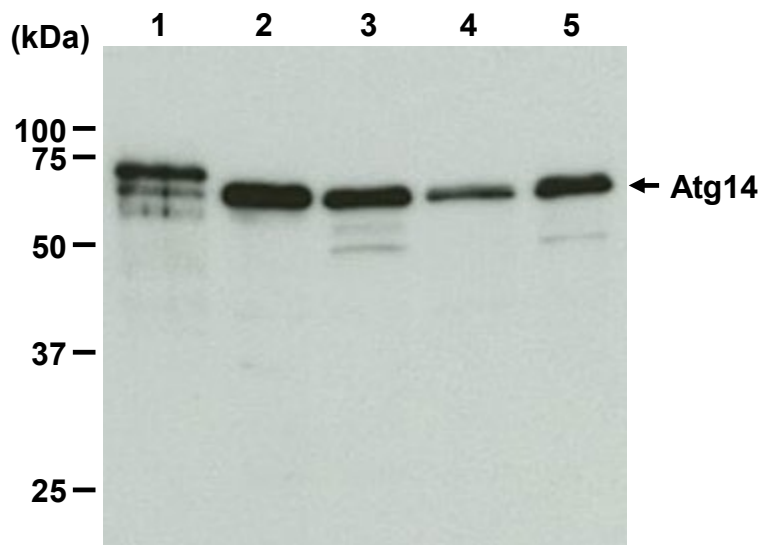
For more information, please visit our website at <https://ruo.mbl.co.jp/>.

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

SDS-PAGE & Western blotting

- 1) Wash 1×10^7 cells 3 times with PBS and suspend them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.).
- 2) Boil the samples for 3 min. and centrifuge. Load 10 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hr. 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.
- 4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
- 6) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 7) Wash the membrane with PBS-T (5 min. x 3 times).
- 8) Incubate the membrane with 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 9) Wash the membrane with PBS-T (5 min. x 3 times).
- 10) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 11) Expose to an X-ray film in a dark room for 5 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; human Atg14 transfectant, HeLa, 293T, A549 and Jurkat)



Western blot analysis of Atg14

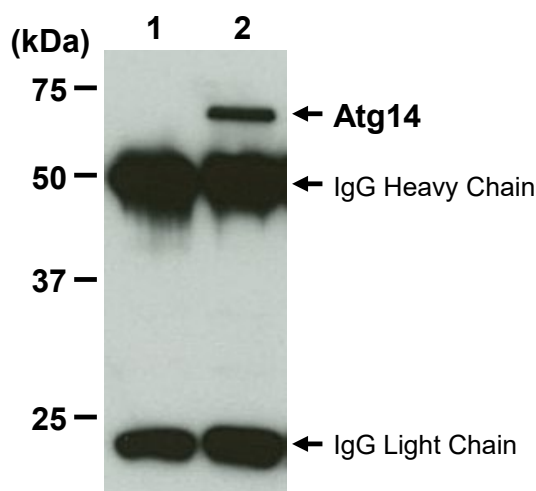
Lane 1: human Atg14/293T
Lane 2: HeLa
Lane 3: 293T
Lane 4: A549
Lane 5: Jurkat

Immunoblotted with M184-3

Immunoprecipitation

- 1) Wash 1×10^7 cells 2 times with PBS and resuspend them with 1 mL of ice-cold Lysis buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40) containing appropriate protease inhibitors, then sonicate briefly (up to 20 sec.).
- 2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
- 3) Mix 20 μ L of 50% protein A agarose beads slurry resuspended in 300 μ L of IP buffer (10 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% NP-40) with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hr. at room temperature.
- 4) Wash the beads 3 times with 1 mL of IP buffer.
- 5) Add 300 μ L of cell lysate (prepared sample of step 2), then incubate with gentle agitation for 1 hr. at room temperature.
- 6) Wash the beads 5 times with 1 mL of Lysis buffer.
- 7) Resuspend the beads in 20 μ L of Laemmli's sample buffer, boil for 2 min. and centrifuge.
- 8) Load 20 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 9) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hr. 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.
- 10) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 11) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
- 12) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 13) Wash the membrane with PBS-T (5 min. x 3 times).
- 14) Incubate the membrane with the 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 15) Wash the membrane with PBS-T (5 min. x 3 times).
- 16) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min.
- 17) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 18) Expose to an X-ray film in a dark room for 5 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Immunoprecipitation; HeLa)



Immunoprecipitation of Atg14 from HeLa

Lane 1: Isotype control (M076-3)

Lane 2: Anti-Atg14 (Human) mAb (M184-3)

Immunoblotted with M184-3