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

Anti-Atg14 (Human) mAb

CODE No. M184-3

CLONALITY Monoclonal
CLONE 4H8
ISOTYPE Mouse IgG2a κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN Human Atg14, 167-404 aa (recombinant)
FORMURATION 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

Western blotting	1 µg/mL for chemiluminescence detection system
Immunoprecipitation	2 µg/300 µL 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)

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MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL http://ruo.mbl.co.jp/
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RELATED PRODUCTS

PM036	Anti-LC3 pAb	[WB, IP, IC, IHC, FCM]
M152-3	Anti-LC3 mAb (4E12)	[WB, IP, IC, FCM, EM]
M186-3	Anti-LC3 mAb (8E10)	[WB]
M186-7	Anti-LC3 mAb-HRP-DirecT (8E10)	
PD014	Anti-LC3 pAb	[WB]
PM045	Anti-p62 (SQSTM1) pAb	
PM066	Anti-p62 C-terminal pAb	
PM066-7	Anti-p62 C-terminal pAb-HRP-DirecT	
M162-3	Anti-p62 (SQSTM1) (Human) mAb (5F2)	
M162-A48	Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor®488 (5F2)	
M162-A59	Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor®594 (5F2)	
M162-A64	Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor®647 (5F2)	
PM074	Anti-Phospho-p62 (SQSTM1) (Ser351) pAb	
M217-3	Anti-Phospho-p62 (SQSTM1) (Ser351) mAb	
D343-3	Anti-Phospho-p62 (SQSTM1) (Ser403) mAb (4F6)	
D344-3	Anti-Phospho-p62 (SQSTM1) (Ser403) mAb (4C8)	
PD017	Anti-Beclin 1 pAb	
PM037	Anti-GABARAP pAb	
M135-3	Anti-GABARAP mAb (1F4)	
PM038	Anti-GATE-16 pAb	
PD041	Anti-Atg2A pAb	
PM034	Anti-Atg3 pAb	
M133-3	Anti-Atg3 mAb (3E8)	
M134-3	Anti-Atg4B mAb (9H5)	
PM050	Anti-Atg5 pAb	
M153-3	Anti-Atg5 mAb (4D3)	
PM039	Anti-Atg7 (Human) pAb	
PD042	Anti-Atg9A pAb	
M151-3	Anti-Atg10 (Human) mAb (5A7)	
M154-3	Anti-Atg12 (Human) mAb (6E5)	
PD036	Anti-Atg13 (Human) pAb	
M183-3	Anti-Atg13 mAb (5G4)	
PD026	Anti-Atg14 pAb	
M184-3	Anti-Atg14 (Human) mAb (4H8)	
PM040	Anti-Atg16L pAb	
M150-3	Anti-Atg16L mAb (1F12)	
M160-3	Anti-UVRAG mAb (1H4)	
PD027	Anti-Rubicon (Human) pAb	
M170-3	Anti-Rubicon (Human) mAb (1H6)	
PD037	Anti-Tel2 pAb	
PM069	Anti-NRF2 pAb	
M200-3	Anti-NRF2 mAb (1F2)	
PM072	Anti-VMP1 pAb	
PM076	Anti-Syntaxin-17 (Human) pAb	
M212-3	Anti-Syntaxin-17 (Human) mAb (2F8)	
M175-3	Anti-α-Tubulin mAb (2F9)	
M175-A48	Anti-α-Tubulin mAb-Alexa Fluor®488 (2F9)	
M175-A59	Anti-α-Tubulin mAb-Alexa Fluor®594 (2F9)	
M175-A64	Anti-α-Tubulin mAb-Alexa Fluor®647 (2F9)	
PM054	Anti-α-Tubulin pAb	
PM054-7	Anti-α-Tubulin pAb-HRP-DirecT	
M176-3	Anti-EEA1 mAb (3C10)	
M176-A48	Anti-EEA1 mAb-Alexa Fluor®488 (3C10)	
M176-A59	Anti-EEA1 mAb-Alexa Fluor®594 (3C10)	
M176-A64	Anti-EEA1 mAb-Alexa Fluor®647 (3C10)	
PM062	Anti-EEA1 pAb	
M178-3	Anti-Calnexin mAb (4F10)	
M178-A48	Anti-Calnexin mAb-Alexa Fluor®488 (4F10)	
M178-A59	Anti-Calnexin mAb-Alexa Fluor®594 (4F10)	
M178-A64	Anti-Calnexin mAb-Alexa Fluor®647 (4F10)	
PM060	Anti-Calnexin pAb	
M181-3	Anti-KDEL mAb (1D5)	
PM059	Anti-KDEL pAb	
M179-3	Anti-GM130 mAb (5G8)	
M179-A48	Anti-GM130 mAb-Alexa Fluor®488 (5G8)	
M179-A59	Anti-GM130 mAb-Alexa Fluor®594 (5G8)	
M179-A64	Anti-GM130 mAb-Alexa Fluor®647 (5G8)	
PM061	Anti-GM130 pAb	
PM063	Anti-COX4 pAb	
PM064	Anti-Lamin B1 pAb	

Kits

8485	Autophagy Ab Sampler Set
8486	Autophagy Watch
PM036-PN	Positive control for anti-LC3 antibody

WB: Western blotting
IP: Immunoprecipitation
IC: Immunocytochemistry
IHC: Immunohistochemistry
FCM: Flow cytometry
EM: Immuno-electron microscopy

Other related antibodies and kits are also available.
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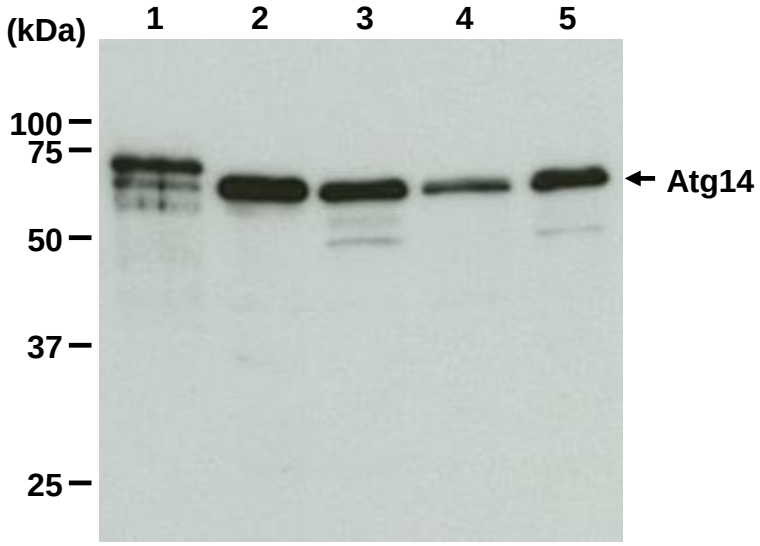
SDS-PAGE & Western blotting

1) Wash 1 x 10^7 cells 3 times with PBS and suspends 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 μL 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/cm^2 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 trasnfectant, HeLa, 293T, A549 and Jurkat)



Western blot analysis of Atg14

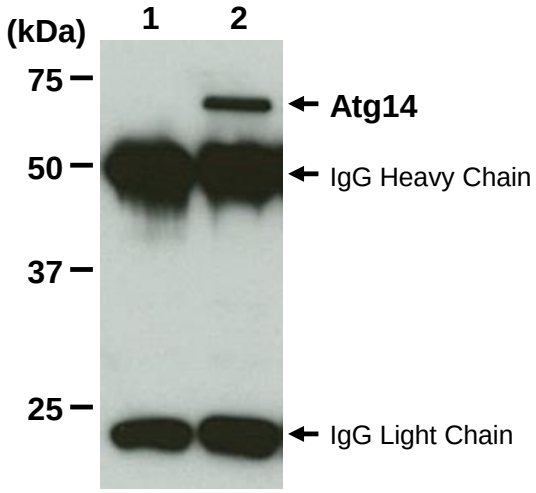
Lane 1: human Atg14/293T
Lane 2: HeLa
Lane 3: 293T
Lane 4: A549
Lane 5: Jurkat

Immunoblotted with M184-3

Immunoprecipitation

1) Wash 1 x 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 µL of 50% protein A agarose beads slurry resuspended in 300 µL 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 µL 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 µL of Laemmli's sample buffer, boil for 2 min. and centrifuge.
8) Load 20 µL 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/cm^2 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