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

Anti-Atg13 mAb

CODE No. M183-3

CLONALITY Monoclonal
CLONE 5G4
ISOTYPE Mouse IgG2a κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN Human Atg13, full-length 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	NIH/3T3, MEF	Rat1	CHO
Reactivity	+	+	+	+

Entrez Gene ID 9776 (Human), 51897 (Mouse), 362164 (Rat), 100753698 (Hamster)

REFERENCES

1) Ganley, I. G., *et al.*, *J. Biol. Chem.* **284**, 12297 (2009)
2) Hosokawa, N., *et al.*, *Mol. Biol. Cell.* **20**, 1981 (2009)
3) Jung, C. H., *et al.*, *Mol. Biol. Cell.* **20**, 1992 (2009)

For more information, please visit our web site **http://ruo.mbl.co.jp/**

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL http://ruo.mbl.co.jp/
e-mail support@mbl.co.jp, TEL 052-238-1904

RELATED PRODUCTS

Antibodies

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)
PD015	Anti-LC3 pAb [IC]
PM045	Anti-p62 (SQSTM1) pAb
PM066	Anti-p62 C-terminal pAb
PM066-7	Anti-p62 C-terminal pAb
M162-3	Anti-p62 (SQSTM1) (Human) mAb (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)
PM054-7	Anti-α-Tubulin pAb-HRP-DirecT
PM053-7	Anti-β-actin pAb-HRP-DirecT

Kits

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

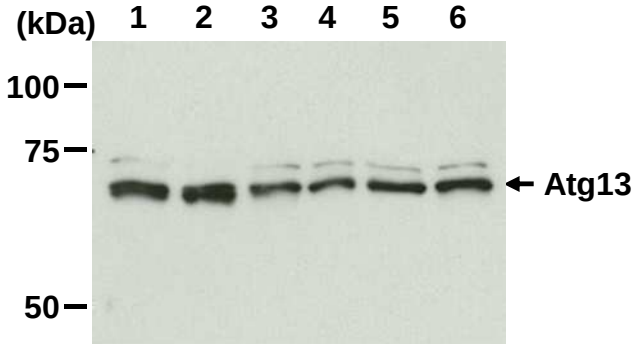
WB: Western blotting
IP: Immunoprecipitation
IC: Immunocytochemistry
IHC: Immunohistochemistry
FCM: Flow cytometry
EM: Immuno-electron microscopy

Other related antibodies and kits are also available.
Please visit our website at http://ruo.mbl.co.jp/

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 20 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 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 of 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 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; HeLa, 293T, NIH3T3, MEF, Rat1 and CHO)

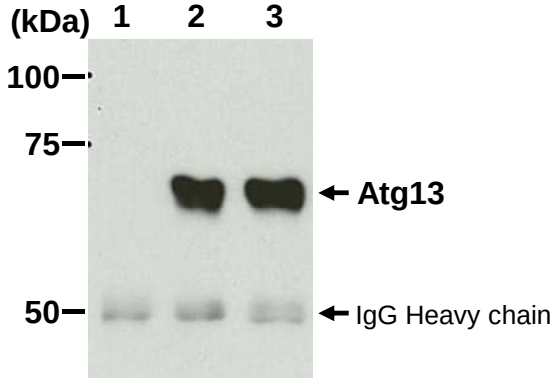


Western blot analysis of Atg13
Lane 1: HeLa
Lane 2: 293T
Lane 3: NIH3T3
Lane 4: MEF
Lane 5: Rat1
Lane 6: CHO
Immunoblotted with M183-3

Immunoprecipitation

1) Wash 1 x 10^7 cells 2 times with PBS and resuspend them with 1 mL of ice-cold Lysis buffer [20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.1% 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) Add the antibody at the amount of suggested in the **APPLICATIONS** into 300 μL of the supernatant. Mix well and incubate with gentle agitation for 60 min. at 4°C.
4) Add 20 μL of 50% protein A agarose resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 min. at 4°C.
5) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 sec.).
6) Resuspend the beads in 20 μL of Laemmli's sample buffer, boil for 2 min. and centrifuge.
7) Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
8) 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.
9) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
10) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
11) Incubate the membrane with 1:500 of Anti-Atg13 (Human) pAb (MBL; code no. PD036) diluted with PBS, pH 7.2 containing 1% skimmed for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
12) Wash the membrane with PBS-T (5 min. x 3 times).
13) Incubate the membrane with 1:10,000 of Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
14) Wash the membrane with PBS-T (5 min. x 3 times).
15) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min.
16) 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 min.
18) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Immunoprecipitation; HeLa)



Immunoprecipitation of Atg13 from HeLa
Lane 1: IP with isotype control (M076-3) (5 μg)
Lane 2: IP with M183-3 (2 μg)
Lane 3: IP with M183-3 (5 μg)
Immunoblotted with PD036