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

# Anti-Atg13

**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)

**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)

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

## RELATED PRODUCTS

| | | |
|---|---|---|
| PD014 | anti-LC3 (polyclonal) | (WB) |
| PD015 | anti-LC3 (polyclonal) | (IC) |
| PM036 | anti-LC3 (polyclonal) | (WB, IP, IC, IHC, FCM) |
| PM046 | anti-LC3 (polyclonal) | (WB, IC) |
| M115-3 | anti-LC3 (51-11) | (WB) |
| M152-3 | anti-LC3 (4E12) | (WB, IP, IC, FCM) |
| M135-3 | anti-GABARAP (1F4) | |
| PM037 | anti-GABARAP (polyclonal) | |
| PM038 | anti-GATE-16 (polyclonal) | |
| PM034 | anti-Atg3 (polyclonal) | |
| M133-3 | anti-Atg3 (3E8) | |
| M134-3 | anti-Atg4B (9H5) | |
| M153-3 | anti-Atg5 (4D3) | |
| PM050 | anti-Atg5 (polyclonal) | |
| PM039 | anti-Atg7 (polyclonal) | |
| M151-3 | anti-Atg10 (5A7) | |
| M154-3 | anti-Atg12 (6E5) | |
| PD036 | anti-Atg13 (polyclonal) | |
| PD026 | anti-Atg14 (polyclonal) | |
| PM040 | anti-Atg16L (polyclonal) | |
| M150-3 | anti-Atg16L (1F12) | |
| M162-3 | anti-p62/SQSTM1 (5F2) | |
| PM045 | anti-p62/SQSTM1 (polyclonal) | |
| M160-3 | anti-UVRAG (1H4) | |
| PD017 | anti-Beclin 1 (polyclonal) | |
| PD027 | anti-Rubicon (polyclonal) | |
| M170-3 | anti-Rubicon (1H6) | |
| PM036-P | Positive control for anti-LC3 antibody | |

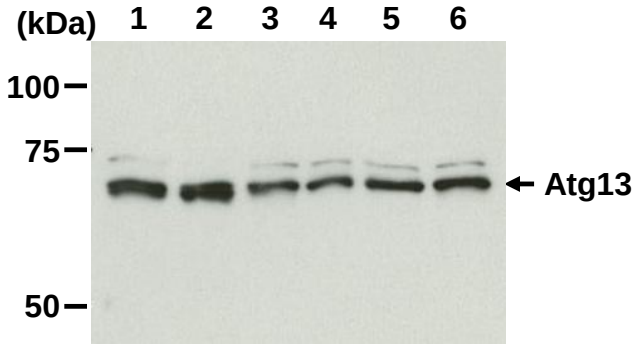
| | |
|---|---|
| M175-3 | anti-α-Tubulin (2F9) |
| PM054 | anti-α-Tubulin (polyclonal) |
| M176-3 | anti-EEA1 (3C10) |
| PM062 | anti-EEA1 (polyclonal) |
| M178-3 | anti-Calnexin (4F10) |
| PM060 | anti-Calnexin (polyclonal) |
| M181-3 | anti-KDEL (1D5) |
| PM059 | anti-KDEL (polyclonal) |
| M179-3 | anti-GM130 (5G8) |
| PM061 | anti-GM130 (polyclonal) |
| PM063 | anti-COX4 (polyclonal) |
| PM064 | anti-Lamin B1 |

WB: Western blotting
IP: Immunoprecipitation
IC: Immunocytochemistry
IHC: Immunohistochemistry
FCM: Flow cytometry

## 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 seconds).
2) 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.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/$cm^2$ 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.
4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for overnight at 4°C.
5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes 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 hour at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T (5 minutes x 3 times).
8) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
9) Wash the membrane with PBS-T (5 minutes x 3 times).
10) Wipe excess buffer on the membrane, and 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.
11) Expose to an X-ray film in a dark room for 3 minute. 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)



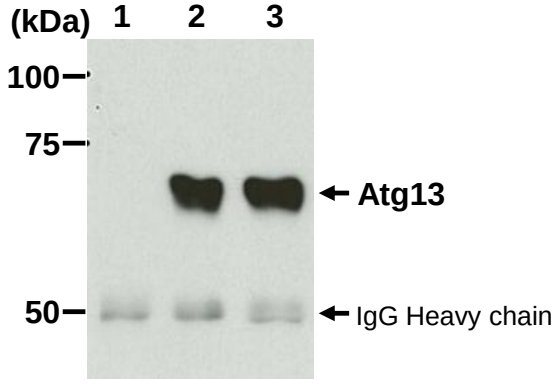

***Western blot analysis of Atg13***

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 seconds).
2) Centrifuge the tube at 12,000 x g for 5 minutes 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 minutes 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 minutes 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 seconds).
6) Resuspend the beads in 20 μL of Laemmli's sample buffer, boil for 2 minutes 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 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.
9) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for overnight at 4°C.
10) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
11) Incubate the membrane with 1:500 of anti-Atg13 polyclonal antibody (MBL; code no. PD036) diluted with PBS, pH 7.2 containing 1% skimmed for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
12) Wash the membrane with PBS-T (5 minutes x 3 times).
13) Incubate the membrane with the 1:10,000 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.
14) Wash the membrane with PBS-T (5 minutes x 3 times).
15) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 minute.
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 minutes.
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