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

# Anti-Atg2A pAb

**CODE No.** PD041

**CLONALITY** Polyclonal
**ISOTYPE** Rabbit Ig, affinity purified
**QUANTITY** 100 µL

**SOURCE** Purified Ig from rabbit serum
**IMMUNOGEN** Human Atg2A, 700-1,400 aa (recombinant)
**FORMULATION** 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 | 1:1,000 for chemiluminescence detection system |
| Immunoprecipitation | 5 µL/300 µL of cell extract from 3 x $10^6$ cells |
| Immunocytochemistry | 1:400 |

**SPECIES CROSS REACTIVITY on WB**

| Species | Human | Mouse | Rat | Hamster |
|---|---|---|---|---|
| Cells | 293T | MEF | PC12 | CHO |
| Reactivity | + | + | + | + |

**Entrez Gene ID** 23130 (Human), 329015 (Mouse), 689688 (Rat), 100758391 (Hamster)

**REFERENCES**

1) Tamura, N., *et al. FEBS Lett.* **591**, 3819-3830 (2017) [WB]
2) Tang, Z., *et al., Cell Death Differ.* **24**, 2127-2138 (2017) [WB]
3) Corcelle-Termeau, E., *et al., Autophagy* **12**, 833-849 (2016) [WB]
4) Velikkakath, A. K., *et al., Mol. Biol. Cell* **23**, 896-909 (2012) [WB, IC]
5) Suzuki, K., *et al., Genes Cell* **12**, 209-218 (2007)
6) Suzuki, K., *et al., EMBO J.* **20**, 5971-5981 (2001)
7) Strømhaug, P. E., *et al., J. Biol. Chem.* **276**, 42422-42435 (2001)
8) Shintani, T., *et al., J. Biol. Chem.* **276**, 30452-30460 (2001)
9) Wang, C. W., *et al., J. Biol. Chem.* **276**, 30442-30451 (2001)

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## 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) | |
| PD014 | Anti-LC3 pAb | [WB] |
| PM045 | Anti-p62 (SQSTM1) pAb | |
| M162-3 | Anti-p62 (SQSTM1) (Human) mAb (5F2) | |
| M162-A48 | Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor®488 mAb (5F2) | |
| M162-A59 | Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor®594 (5F2) | |
| M162-A64 | Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor®647 (5F2) | |
| PM066 | Anti-p62 C-terminal pAb | |
| D343-3 | Anti-Phospho-p62 (SQSTM1) (Ser403) mAb (4F6) | |
| D344-3 | Anti-Phospho-p62 (SQSTM1) (Ser403) mAb (4C8) | |
| PM074 | Anti-Phospho-p62 (SQSTM1) (Ser351) pAb | |
| M217-3 | Anti-Phospho-p62 (SQSTM1) (Ser351) mAb (5D5) | |
| PD017 | Anti-Beclin 1 pAb | |
| PM037 | Anti-GABARAP pAb | |
| M135-3 | Anti-GABARAP mAb (1F4) | |
| PM038 | Anti-GATE-16 pAb | |
| PD041 | Anti-Atg2A 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) | |
| 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) | |
| PM072 | Anti-VMP1 pAb | |
| PM076 | Anti-Syntaxin-17 (Human) pAb | |
| M212-3 | Anti-Syntaxin-17 (Human) mAb (2F8) | |
| PM069 | Anti-NRF2 pAb | |
| M200-3 | Anti-NRF2 mAb (1F2) | |
| M224-3 | Anti-KEAP1 mAb (KP1) | |
| M230-3 | Anti-Parkin mAb (Par6) | |

Kits

| | |
|---|---|
| 8485 | Autophagy Ab Sampler Set |
| 8486 | Autophagy Watch |
| CY-7055 | CycLex® Total p62 ELISA Kit |
| CY-7056 | CycLex® Phospho-p62 Ser349 ELISA Kit |
| CY-7057 | CycLex® Phospho-p62 Ser403 ELISA Kit |
| PM036-PN | Positive control for anti-LC3 antibody |

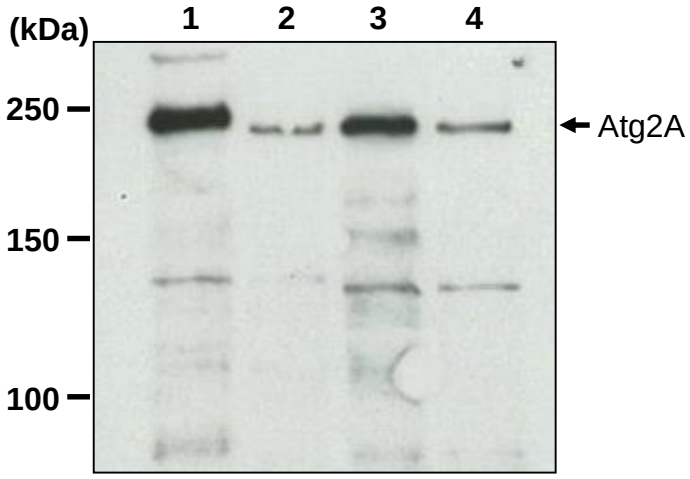
WB: Western blotting
IP: Immunoprecipitation
IC: Immunocytochemistry
IHC: Immunohistochemistry
FCM: Flow cytometry
EM: Immuno-electron microscopy

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) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
3) Boil the samples for 3 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
4) 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.
5) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
6) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) (5 min. x 3 times).
7) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
8) Wash the membrane with PBS-T (5 min. x 3 times).
9) Incubate the membrane with 1:10,000 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.
10) Wash the membrane with PBS-T (5 min. x 3 times).
11) 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.
12) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; 293T, MEF, PC12 and CHO)



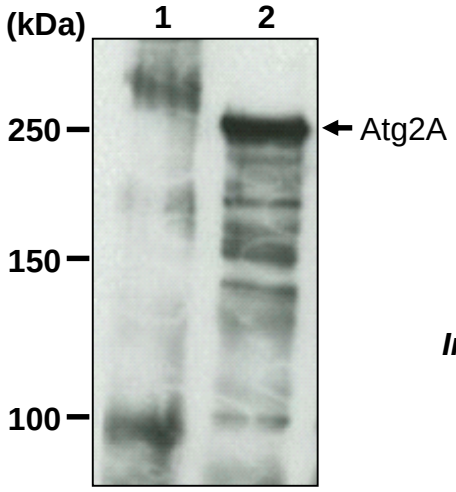

***Western blot analysis of Atg2A***

Lane 1: 293T
Lane 2: MEF
Lane 3: PC12
Lane 4: CHO
Immunoblotted with PD041

## 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. (The amount of antibody will depend on the conditions.)
4) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
5) Resuspend the beads with 1 mL of IP buffer.
6) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
7) Repeat steps 4)-6) 2 times.
8) Add 300 µL of cell lysate (prepared sample from step 2)), then incubate with gentle agitation for 1 hr. at room temperature.
9) Wash the beads 5 times with 1 mL of Lysis buffer.
10) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3 min. and centrifuge.
11) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
12) 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.
13) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
14) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) (5 min. x 3 times).
15) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
16) Wash the membrane with PBS-T (5 min. x 3 times).
17) Incubate the membrane with 1:10,000 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.
18) Wash the membrane with PBS-T (5 min. x 3 times).
19) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min.
20) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
21) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; 293T)

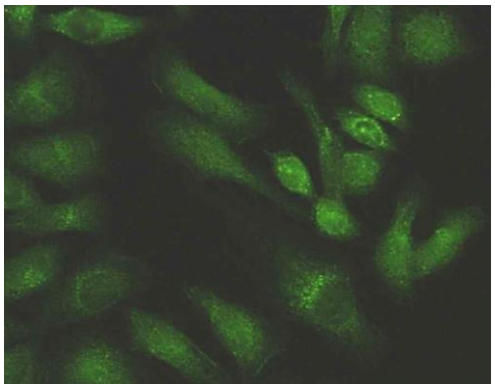



***Immunoprecipitation of Atg2A from 293T***
Lane 1: IP with Normal Rabbit IgG (PM035)
Lane 2: IP with PD041
Immunoblotted with PD041

## Immunocytochemistry

1) Spread the cells on a glass slide, then incubate in a $CO_2$ incubator for one night.
2) Remove the culture supernatant by careful aspiration.
3) To obtain serum-starved conditions, culture the cells with Hanks' balanced salt solution or DMEM for 2-4 hr. at 37°C.
4) Fix the cells by immersing the slide in 4% paraformaldehyde (PFA)/PBS for 10 min. at room temperature (20~25°C).
5) Wash the slide 2 times with PBS.
6) Immerse the slide in 100 μg/mL of Digitonin in PBS for 10 min. at room temperature.
7) Wash the slide 2 times with PBS.
8) Add 200 μL of the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hr. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
9) Wash the slide 2 times with PBS.
10) Add 200 μL of 1:500 anti-IgG (Rabbit)-Alexa Fluor®488 (Invitrogen; code no. A11008) diluted with PBS onto the cells. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
11) Wash the slide 2 times with PBS.
12) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; starved HeLa)



***Immunocytochemical detection of Atg2A in starved HeLa***