

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

 **My select** sampler set

## Anti-Atg2A pAb

**CODE No.** PD041MS

**CLONALITY** Polyclonal  
**ISOTYPE** Rabbit Ig, affinity purified  
**QUANTITY** 20 µL

**SOURCE** Purified Ig from rabbit serum  
**IMMUNOGEN** Human Atg2A, 700 aa-1,400 aa (recombinant)  
**FORMURATION** 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<sup>6</sup> cells  
Immunocytochemistry 1:400

### SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cell	293T	MEF	PC12	CHO
Reactivity	+	+	+	+

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

**REFERENCES**

- 1) Velikkakath, A. K., *et al.*, *Mol. Biol. Cell* **23**, 896-909 (2012) [WB, IC]
- 2) Suzuki, K., *et al.*, *Genes Cell* **12**, 209-218 (2007)
- 3) Suzuki, K., *et al.*, *EMBO J.* **20**, 5971-5981 (2001)
- 4) Strømhaug, P. E., *et al.*, *J. Biol. Chem.* **276**, 42422-42435 (2001)
- 5) Shintani, T., *et al.*, *J. Biol. Chem.* **276**, 30452-30460 (2001)
- 6) 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]
PD014	Anti-LC3 pAb	[WB]
PD015	Anti-LC3 pAb	[IC]
PM046	Anti-LC3 pAb	[WB, IC]
M115-3	Anti-LC3 mAb (51-11)	[WB]
PM045	Anti-p62 (SQSTM1) pAb	
M162-3	Anti-p62 (SQSTM1) (Human) mAb (5F2)	
M162-A48	Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor <sup>®</sup> 488 (5F2)	
M162-A59	Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor <sup>®</sup> 594 (5F2)	
M162-A64	Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor <sup>®</sup> 647 (5F2)	
PM066	Anti-p62 C-terminal pAb	
PD017	Anti-Becclin 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)	
PM069	Anti-NRF2 pAb	
M200-3	Anti-NRF2 mAb (1F2)	
PD037	Anti-Tel2 pAb	
PM072	Anti-VMP1 pAb	

M175-3	Anti- $\alpha$ -Tubulin mAb (2F9)	
M175-A48	Anti- $\alpha$ -Tubulin mAb-Alexa Fluor <sup>®</sup> 488 (2F9)	
M175-A59	Anti- $\alpha$ -Tubulin mAb-Alexa Fluor <sup>®</sup> 594 (2F9)	
M175-A64	Anti- $\alpha$ -Tubulin mAb-Alexa Fluor <sup>®</sup> 647 (2F9)	
PM054	Anti- $\alpha$ -Tubulin pAb	
PM054-7	Anti- $\alpha$ -Tubulin pAb-HRP-Direct	
M176-3	Anti-EEA1 mAb (3C10)	
M176-A48	Anti-EEA1 mAb-Alexa Fluor <sup>®</sup> 488 (3C10)	
M176-A59	Anti-EEA1 mAb-Alexa Fluor <sup>®</sup> 594 (3C10)	
M176-A64	Anti-EEA1 mAb-Alexa Fluor <sup>®</sup> 647 (3C10)	
PM062	Anti-EEA1 pAb	
M178-3	Anti-Calnexin mAb (4F10)	
M178-A48	Anti-Calnexin mAb-Alexa Fluor <sup>®</sup> 488 (4F10)	
M178-A59	Anti-Calnexin mAb-Alexa Fluor <sup>®</sup> 594 (4F10)	

M178-A64	Anti-Calnexin mAb-Alexa Fluor <sup>®</sup> 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 <sup>®</sup> 488 (5G8)	
M179-A59	Anti-GM130 mAb-Alexa Fluor <sup>®</sup> 594 (5G8)	
M179-A64	Anti-GM130 mAb-Alexa Fluor <sup>®</sup> 647 (5G8)	
PM061	Anti-GM130 pAb	
PM063	Anti-COX4 pAb	
PM064	Anti-Lamin B1 pAb	

Kits

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

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

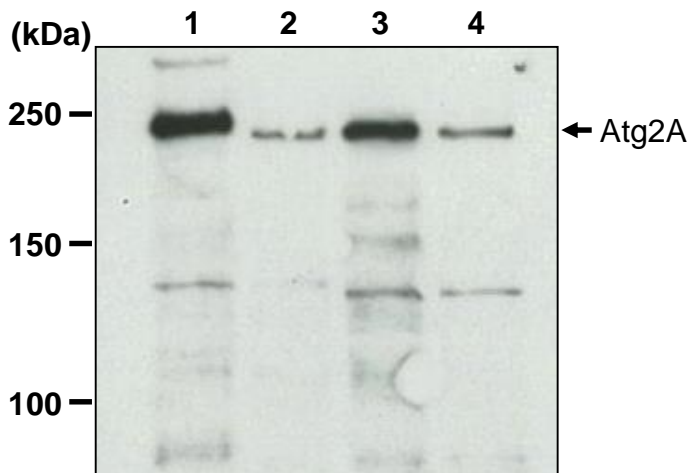
Other related antibodies and kits are also available.

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### **SDS-PAGE & Western blotting**

- 1) Wash  $1 \times 10^7$  cells 3 times with PBS and suspend them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 20 sec.).
- 2) Centrifuge the tube at  $12,000 \times g$  for 5 min. at  $4^\circ\text{C}$  and transfer the supernatant to another tube.
- 3) Boil the samples for 3 min. and centrifuge. Load  $10 \mu\text{L}$  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  $1 \text{ mA}/\text{cm}^2$  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) for overnight at  $4^\circ\text{C}$ .
- 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 the 1:10,000 anti-IgG (Rabbit)-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

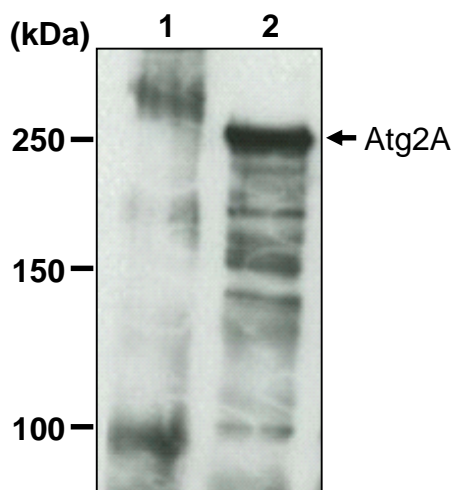
Lane 4: CHO

Immunoblotted with PD041

### Immunoprecipitation

- 1) Wash  $1 \times 10^7$  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  $\mu$ L of 50% protein A agarose beads slurry resuspended in 300  $\mu$ 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 gently agitation for 1 hr. at room temperature. (The amount of antibody will depend on the conditions.)
- 4) Wash the beads 3 times with 1 mL of IP buffer.
- 5) Add 300  $\mu$ L of cell lysate (prepared sample from 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  $\mu$ L of Laemmli's sample buffer, boil for 3 min. and centrifuge.
- 8) Load 10  $\mu$ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
- 9) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> 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) for 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 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.)
- 13) Wash the membrane with PBS-T (5 min. x 3 times).
- 14) Incubate the membrane with the 1:10,000 anti-IgG (Rabbit)-HRP (MBL; code no. 458) 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 1 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; 293T)



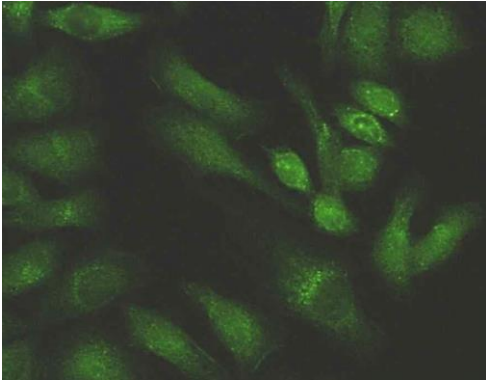
### ***Immunoprecipitation of Atg2A from 293T***

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<sub>2</sub> 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<sup>®</sup>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***