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
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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The descriptions of the following protocols are examples. Each user should determine the appropriate condition.
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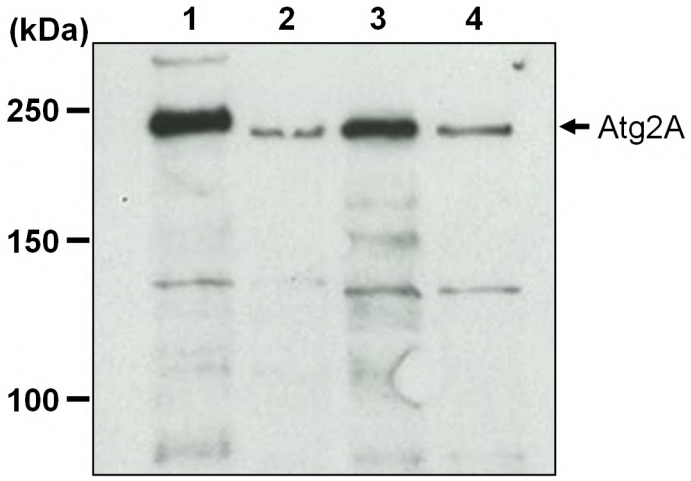
SDS-PAGE & Western blotting

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 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)

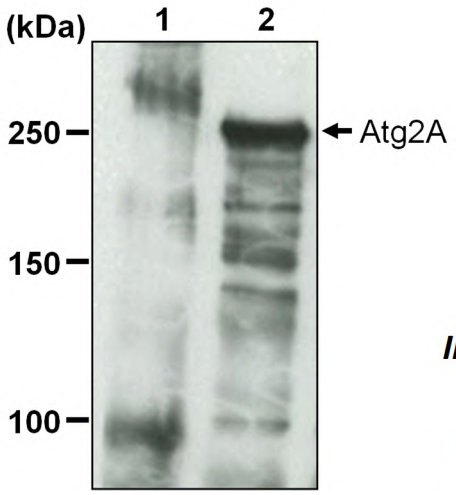


Western blot analysis of Atg2A
Lane 1: 293T
Lane 2: MEF
Lane 3: PC12
Lane 4: CHO
Immunoblotted with PD041

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)

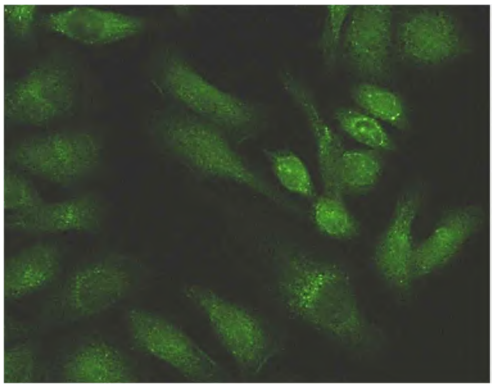


Immunoprecipitation of Atg2A from 293T
Lane 1: IP with Normal Rabbit IgG (PM035)
Lane 2: IP with PD041
Immunoblotted with PD041

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