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

My select sampler set

Anti-Atg9A pAb

CODE No.	PD042MS
CLONALITY	Polyclonal
ISOTYPE	Rabbit Ig, affinity purified
QUANTITY	20 μL
SOURCE	Purified Ig from rabbit serum
IMMUNOGEN	Mouse Atg9A, 506-839 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	1:500
Immunoprecipitation	2.5 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 79065 (Human), 245860 (Mouse), 363254 (Rat), 100774022 (Hamster)

REFERENCES

1) Itakura, E., *et al.*, *J. Cell Sci.* **125**, 1488-1499 (2012) [IC]
2) Young, A. R., *et al.*, *J. Cell Sci.* **119**, 3888-3900 (2006)
3) Yamada, T., *et al.*, *J. Bio. Chem.* **280**, 18283-18290 (2005)

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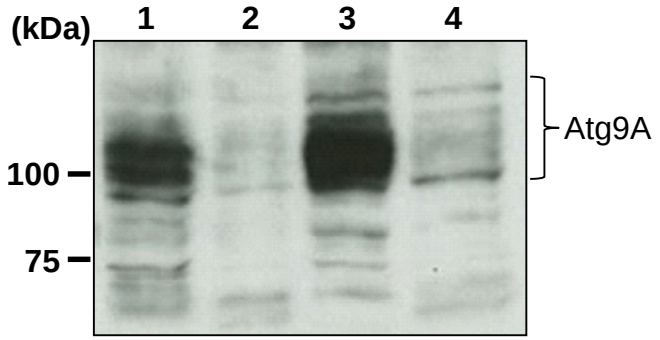
SDS-PAGE & Western blotting

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) Heating the samples at 55°C for 5 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% methanol). 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).
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).
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).
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 blotting analysis of Atg9A

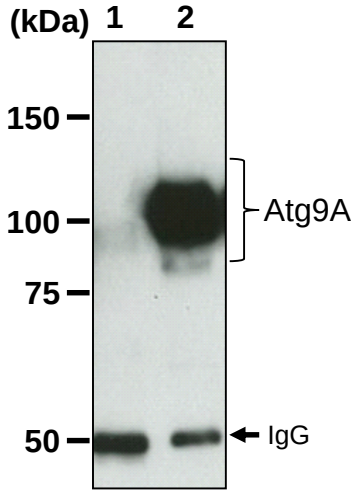
Lane 1: 293T
Lane 2: MEF
Lane 3: PC12
Lane 4: CHO

Immunoblotted with Anti-Atg9A pAb (MBL, code no. PD042)

Immunoprecipitation

1) Wash 1 x 10^7 cells twice 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) Wash the beads 3 times with 1 mL of IP buffer.
5) Add 300 µL of cell lysate (prepared sample from step 2)), then incubate with gentle agitation for 1 hr. at room temperature.
6) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant using a pipettor without disturbing the beads.
7) Resuspend the beads with 1 mL of cold Lysis buffer.
8) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant.
9) Repeat steps 7)-8) 4 times.
10) Resuspend the beads in 20 µL of Laemmli's sample buffer, heat it at 55°C for 5 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% methanol). 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).
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).
17) Incubate the membrane with the 1:1,000 Rabbit TrueBlot® anti-Rabbit IgG-HRP (Rockland, code no. 18-8816-33) 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).
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 Atg9A from 293T

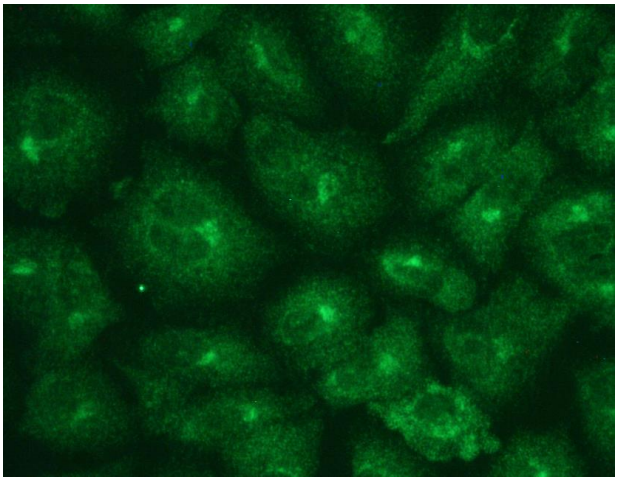
Lane 1: IP with Normal Rabbit IgG (MBL, code no. PM035)
Lane 2: IP with Anti-Atg9A pAb (MBL, code no. PD042)

Immunoblotted with Anti-Atg9A pAb (MBL, code no. PD042)

Immunocytochemistry

1) Spread the cells on a glass slide, then incubate in a CO_2 incubator overnight.
2) Remove the culture supernatant by careful aspiration.
3) Fix the cells by immersing the slide in 4% paraformaldehyde/PBS for 10 min. at room temperature (20~25°C).
4) Wash the slide twice with PBS.
5) Immerse the slide in 100 μg/mL of Digitonin in PBS for 10 min. at room temperature.
6) Wash the slide twice with PBS.
7) 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.)
8) Wash the slide twice with PBS.
9) Add 200 μL of 1:500 anti-IgG (Rabbit)-Alexa Fluor®488 (Thermo Fisher Scientific, code no. A-11008) diluted with PBS onto the cells. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
10) Wash the slide twice with PBS.
11) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; A549)



Immunocytochemical detection of Atg9A in A549