

 My select sampler set

## Anti-Atg13 mAb

<b>CODE No.</b>	M183-3MS
<b>CLONALITY</b>	Monoclonal
<b>CLONE</b>	5G4
<b>ISOTYPE</b>	Mouse IgG2a $\kappa$
<b>QUANTITY</b>	20 $\mu$ L, 1 mg/mL
<b>SOURCE</b>	Purified IgG from hybridoma supernatant
<b>IMMUNOGEN</b>	Human Atg13, full-length recombinant
<b>FORMURATION</b>	1 mg/mL in PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
<b>STORAGE</b>	This antibody solution is stable for one year from the date of purchase when stored at -20°C.

### APPLICATIONS-CONFIRMED

<u>Western blotting</u>	1 $\mu$ g/mL for chemiluminescence detection system
<u>Immunoprecipitation</u>	2 $\mu$ g/300 $\mu$ L of cell extract from $3 \times 10^6$ 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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**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® 488 (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	
PD017	Anti-Beclin 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	

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/>

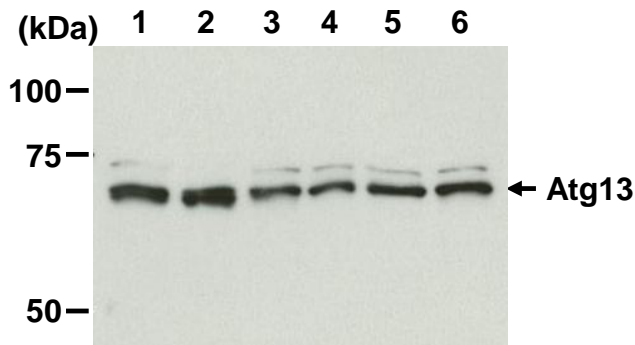
Kits

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

**SDS-PAGE & Western blotting**

- 1) Wash  $1 \times 10^7$  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  $\mu$ L 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<sup>2</sup> 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)



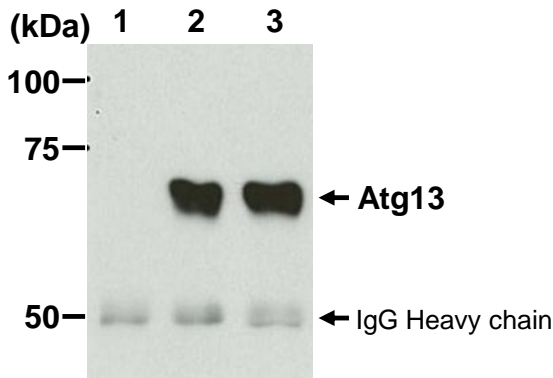
**Western blot analysis of Atg13**

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  $1 \times 10^7$  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  $\mu$ L of the supernatant. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
- 4) Add 20  $\mu$ L 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  $\mu$ L of Laemmli's sample buffer, boil for 2 minutes and centrifuge.
- 7) Load 10  $\mu$ L 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<sup>2</sup> 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  $\mu$ g)  
Lane 2: IP with M183-3 (2  $\mu$ g)  
Lane 3: IP with M183-3 (5  $\mu$ g)  
Immunoblotted with PD036