

## Anti-Syntaxin-17 (Human) pAb

<b>CODE No.</b>	PM076
<b>CLONALITY</b>	Polyclonal
<b>ISOTYPE</b>	Rabbit Ig, affinity purified
<b>QUANTITY</b>	100 µL
<b>SOURCE</b>	Purified IgG from rabbit serum
<b>IMMUNOGEN</b>	Human Syntaxin-17, recombinant protein
<b>FORMURATION</b>	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>	1:1,000 for chemiluminescence detection system
<u>Immunoprecipitation</u>	2.5 µL/sample
<u>Immunocytochemistry</u>	1:2,000

### SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cells	Jurkat, A549, HeLa	NIH/3T3	NRK	Not tested
Reactivity	+	-	-	

**Entrez Gene ID** 55014 (Human)

**REFERENCES**  
1) Button, R. W., *et al.*, *J. Biol. Chem.* (2017) In press. [WB]  
2) Itakura, E., *et al.*, *Cell* **151**, 1256–1269 (2012)

For more information, please visit our web site <http://ruo.mbl.co.jp/>

## RELATED PRODUCTS

### Antibodies

PM076	Anti-Syntaxin-17 (Human) pAb
K0117-3	Anti-Syntaxin-1 mAb (HPC-1)
K0118-3	Anti-Syntaxin-6 mAb (3D10)
K0119-3	Anti-Syntaxin-7 (Human) mAb (Syn7.1C3)
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 (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
PM066-7	Anti-p62 C-terminal pAb-HRP-Direct
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
PD017	Anti-Bec1 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® 488 (2F9)
M175-A59	Anti- $\alpha$ -Tubulin mAb-Alexa Fluor® 594 (2F9)
M175-A64	Anti- $\alpha$ -Tubulin mAb-Alexa Fluor® 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® 488 (3C10)
M176-A59	Anti-EEA1 mAb-Alexa Fluor® 594 (3C10)
M176-A64	Anti-EEA1 mAb-Alexa Fluor® 647 (3C10)
PM062	Anti-EEA1 pAb
M178-3	Anti-Calnexin mAb (4F10)
M178-A48	Anti-Calnexin mAb-Alexa Fluor® 488 (4F10)
M178-A59	Anti-Calnexin mAb-Alexa Fluor® 594 (4F10)
M178-A64	Anti-Calnexin mAb-Alexa Fluor® 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® 488 (5G8)
M179-A59	Anti-GM130 mAb-Alexa Fluor® 594 (5G8)
M179-A64	Anti-GM130 mAb-Alexa Fluor® 647 (5G8)
PM061	Anti-GM130 pAb
PM063	Anti-COX4 pAb
PM064	Anti-Lamin B1 pAb

### Kits

8485	Autophagy Ab Sampler Set
8486	Autophagy Watch
PM036-PN	Positive control for anti-LC3 antibody

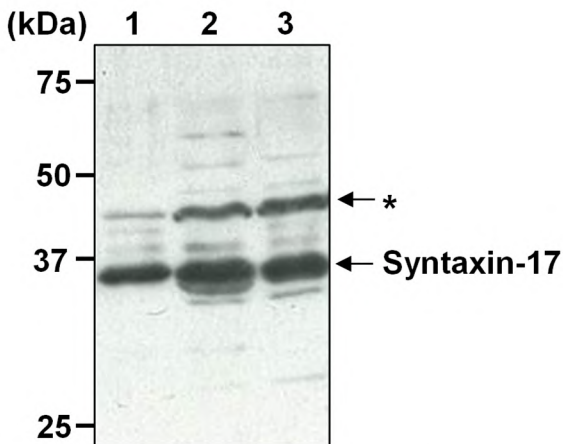
WB: Western blotting  
IP: Immunoprecipitation  
IC: Immunocytochemistry  
IHC: Immunohistochemistry  
FCM: Flow cytometry  
EM: Immuno-electron microscopy

Other related antibodies and kits are also available.  
Please visit our web site at <http://ruo.mbl.co.jp>

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

- 1) Wash  $1 \times 10^7$  cells 3 times with PBS and suspend with 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 5 min. and centrifuge. Load 10  $\mu$ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 4) 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.
- 5) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 6) 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.)
- 7) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
- 8) Incubate the membrane with the 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.
- 9) Wash the membrane with PBS-T (5 min. x 3 times)
- 10) Wipe excess buffer on the membrane, 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.
- 11) Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Jurkat, A549 and HeLa)



\* Non-specific band

### ***Western blot analysis of Syntaxin-17***

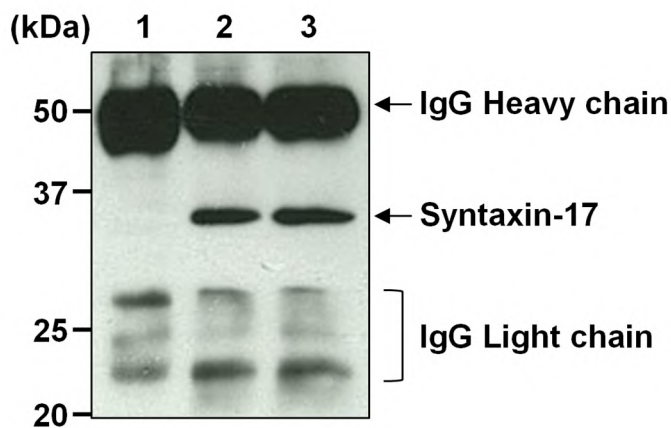
Lane 1: Jurkat  
Lane 2: A549  
Lane 3: HeLa

Immunoblotted with Anti-Syntaxin-17 (Human) pAb (PM076)

### Immunoprecipitation

- 1) Resuspend  $1 \times 10^7$  cells with 1 mL of ice-cold Extraction buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% NP-40) containing appropriate protease inhibitors.
- 2) Centrifuge the tube at 12,000 x g for 10 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 400  $\mu$ L of IP buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40) with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 30 min. at 4°C.
- 4) Wash the beads 1 time 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 4°C.
- 6) Wash the beads 4 times with 1 mL of Extraction buffer.
- 7) Resuspend the beads in 20  $\mu$ L of Laemmli's sample buffer, boil for 5 min. and centrifuge.
- 8) Load 10  $\mu$ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% 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) overnight at 4°C.
- 11) 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.)
- 12) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
- 13) Incubate the membrane with the 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.
- 14) Wash the membrane with PBS-T (5 min. x 3 times)
- 15) Wipe excess buffer on the membrane, 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.  
Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; Jurkat)



### **Immunoprecipitation of Syntaxin-17 from Jurkat**

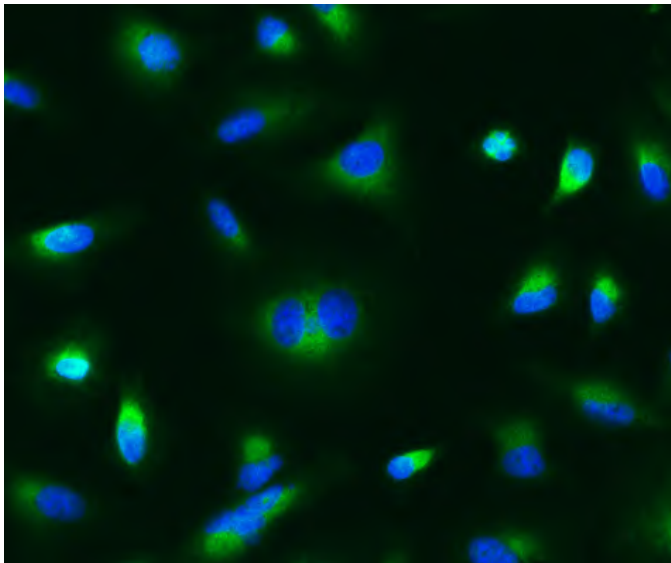
- Lane 1: IP with 1  $\mu$ L of Normal Rabbit IgG (MBL; code no. PM035)
- Lane 2: IP with 2.5  $\mu$ L of Anti-Syntaxin-17 (Human) pAb (PM076)
- Lane 3: IP with 5  $\mu$ L of Anti-Syntaxin-17 (Human) pAb (PM076)

Immunoblotted with PM076

### **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) Wash the slide 2 times with PBS.
- 4) Fix the cells with 4% paraformaldehyde (PFA)/PBS for 10 min. at room temperature (20~25°C).
- 5) Wash the slide 2 times with PBS.
- 6) Permeabilize the cells with 100 µg/mL of Digitonin/PBS for 10 min. at room temperature.
- 7) Wash the slide 2 times with PBS.
- 8) Tip off PBS and add 200 µL of the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells. 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 Alexa Fluor® 488 Goat Anti-rabbit IgG (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) Counterstain with DAPI for 5 minutes at room temperature.
- 13) Wash the glass slide 2 times with PBS.
- 14) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry.
- 15) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; A549)



### ***Immunocytochemical detection of Syntaxin-17 in A549***

Green: Anti-Syntaxin-17 (Human) pAb (PM076)  
Blue: DAPI