

MONOCLONAL ANTIBODY

# Anti-Rubicon (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M170-3	1H6	Mouse IgG2a $\kappa$	100 $\mu$ L	1 mg/mL

**BACKGROUND:** Autophagy is a process of intracellular bulk degradation in which cytoplasmic components including organelles are sequestered within double-membrane vesicles that deliver the contents to the lysosome/vacuole for degradation. Rubicon was identified as Beclin1 interacting protein. Three distinct Beclin1 complexes exist in cells, one of the complexes including Rubicon (Beclin1, hVps34, hVps15, UVRAG, Rubicon) down regulates the process of autophagosome maturation and endocytosis.

**SOURCE:** This antibody was purified from hybridoma (clone 1H6) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with the recombinant human Rubicon (722-972 aa).

**FORMULATION:** 100  $\mu$ g IgG in 100  $\mu$ L volume of 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  $-20^{\circ}\text{C}$ .

**REACTIVITY:** This antibody reacts with human Rubicon on Western blotting.

**APPLICATIONS:**

Western blotting: 1  $\mu$ g/mL for chemiluminescence detection system

Immunoprecipitation: Not recommended

Immunohistochemistry: Can be used

Immunocytochemistry: Not tested

Flow cytometry: Not tested

Detailed procedure is provided in the following **PROTOCOLS**.

**SPECIES CROSS REACTIVITY:**

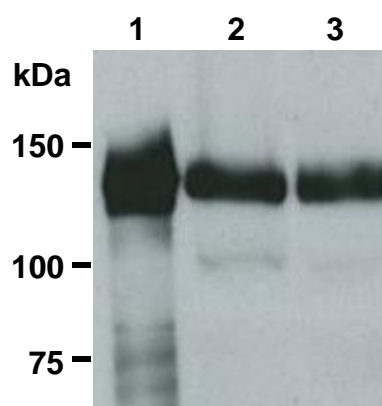
Species	Human	Mouse	Rat
Cells	HeLa, A549, transfectant	NIH/3T3, WR19L	Not tested
Reactivity on WB	+	-	

**REFERENCES:**

- 1) Matsunaga, K., *et al.*, *Nat. Cell Biol.* **11**, 385-396 (2009)
- 2) Zhong, Y., *et al.*, *Nat. Cell Biol.* **11**, 468-476 (2009)

**INTENDED USE:**

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



**Western blot analysis of Rubicon expression on Flag tagged Rubicon transfectant (1), HeLa (2) and A549 (3) using M170-3.**

**PROTOCOLS:**

**SDS-PAGE & Western Blotting**

- 1) Wash cells (approximately  $1 \times 10^7$  cells) 3 times with PBS and resuspend them in 1 mL of Laemmli's sample buffer.
- 2) Boil the samples for 3 minutes and centrifuge. Load 20  $\mu$ L of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out 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 7.5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at  $4^{\circ}\text{C}$ .
- 5) Incubate the membrane for 1 hour at room temperature with primary antibody diluted with PBS (pH 7.2) containing 1% skimmed milk as suggested in the **APPLICATIONS**. (The concentration of antibody will depend on the conditions.)
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 7) Incubate the membrane with 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed

- milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 8) Wash the membrane with PBS-T (5 minutes x 3 times).
  - 9) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
  - 10) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
  - 11) Expose the membrane onto an X-ray film in a dark room for 10 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive control for Western blotting; HeLa, A549, transfectant)

PM069	Anti-NRF2 pAb
M200-3	Anti-NRF2 mAb (1F2)
M224-3	Anti-KEAP1 mAb (KP1)
M230-3	Anti-Parkin mAb (Par6)

#### Kits

8485	Autophagy Ab Sampler Set
8486	Autophagy Watch
CY-7055	CycLex <sup>®</sup> Total p62 ELISA Kit
CY-7056	CycLex <sup>®</sup> Phospho-p62 Ser349 ELISA Kit
CY-7057	CycLex <sup>®</sup> Phospho-p62 Ser403 ELISA Kit
PM036-PN	Positive control for anti-LC3 antibody

#### **RELATED PRODUCTS:**

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 <sup>®</sup> 488 mAb (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	
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	
M217-3	Anti-Phospho-p62 (SQSTM1) (Ser351) mAb (5D5)	
PD017	Anti-Becn1 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)	
PD037	Anti-Tel2 pAb	
PM072	Anti-VMP1 pAb	
PM076	Anti-Syntaxin-17 (Human) pAb	
M212-3	Anti-Syntaxin-17 (Human) mAb (2F8)	

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/>