

 **My select** sampler set

Early Endosome Marker

## Anti-EEA1 pAb

**Code No.**  
PM062MS

**Quantity**  
20  $\mu$ L

**Form**  
Affinity Purified

**BACKGROUND:** The early endosome is a cellular compartment inside eukaryotic cells. It is receiving endocytosed material and sorting them for vesicular transport to late endosomes and lysosomes or for recycling to the plasma membrane. EEA1 (Early Endosome Antigen1) is a 170 kDa coiled-coil protein, which is required for vesicular transport of proteins through early endosomes. It binds membrane lipids through its FYVE domain.

**SOURCE:** This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with the synthetic peptide corresponding to N-terminus of human EEA1.

**FORMULATION:** 20  $\mu$ 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 EEA1 for Western blotting, Immunoprecipitation and Immunocytochemistry.

### APPLICATIONS:

Western blotting; 1:1,000 for chemiluminescence detection system

Immunoprecipitation; 2  $\mu$ L/300  $\mu$ L of cell extract from  $1 \times 10^7$  cells

Immunohistochemistry; Not tested

Immunocytochemistry; 1:200

Flow cytometry; Not tested

Detailed procedures are provided in the following **PROTOCOLS**.

### SPECIES CROSS REACTIVITY:

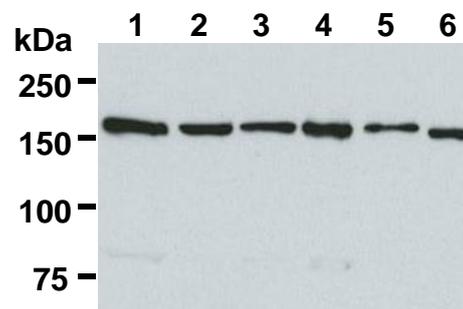
Species	Human	Mouse	Rat
Cells	HeLa, 293T, A549	NIH/3T3, MEF	NRK
Reactivity on WB	+	+	+

### INTENDED USE:

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

### REFERENCES:

- 1) Gaullier, J. M., *et al.*, *J. Biol. Chem.* **275**, 24595-24600 (2000)
- 2) Mu, F. T., *et al.*, *J. Biol. Chem.* **270**, 13503-13511 (1995)



**Western blot analysis of EEA1 expression on HeLa (1), 293T (2), A549 (3), NIH/3T3 (4), MEF (5) and NRK (6) using PM062.**

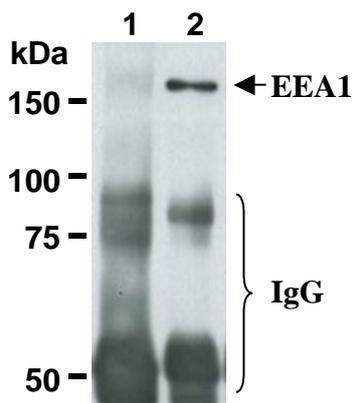
### PROTOCOLS:

#### 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 2 minutes and centrifuge. Load 10  $\mu$ 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 10% 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 1% skimmed milk (in PBS, pH 7.2) 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 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.
- 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 3 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive controls for Western blotting; HeLa, 293T, A549, NIH/3T3, MEF, NRK)



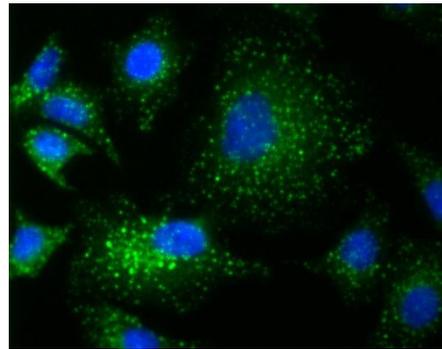
**Immunoprecipitation of EEA1 from HeLa with normal rabbit IgG (1) or PM062 (2).** After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with PM062.

#### Immunoprecipitation

- 1) Wash cells (approximately  $1 \times 10^7$  cells) 3 times with PBS and resuspend them in 1 mL of cold Lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% NP-40) containing protease inhibitors at appropriate concentrations. Incubate it at 4°C with rotating for 30 minutes; thereafter, briefly sonicate the mixture (up to 10 seconds).
- 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
- 3) Add primary antibody as suggested in the **APPLICATIONS** into 300  $\mu$ L of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20  $\mu$ L of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
- 4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
- 5) Resuspend the beads in 20  $\mu$ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10  $\mu$ L/lane for the SDS-PAGE analysis.

(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; HeLa)



**Immunocytochemical detection of EEA1 in A549 with PM062.**  
Green: anti-EEA1  
Blue: DAPI

#### Immunocytochemistry

- 1) Culture the cells in the appropriate condition on a glass slide. (for example, spread  $1 \times 10^4$  cells for one slide, then incubate in a CO<sub>2</sub> incubator for one night.)
- 2) Wash the glass slide 2 times with PBS.
- 3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde for 10 minutes at room temperature.
- 4) Wash the glass slide 3 times with PBS.
- 5) Immerse the slide in PBS containing 0.2% Triton X-100 for 10 minutes at room temperature.
- 6) Wash the glass slide 2 times with PBS.
- 7) Add the primary antibody diluted with 2% FCS/PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hour at room temperature (Optimization of antibody concentration or incubation condition is recommended if necessary).
- 8) Wash the glass slide 2 times with PBS.
- 9) Add 100  $\mu$ L of 1:500 Alexa Fluor<sup>®</sup> 488 conjugated anti-rabbit IgG (Invitrogen; code no. A110374) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
- 10) Wash the glass slide 3 times with PBS.
- 11) Counter stain with DAPI for 5 minutes at room temperature.
- 12) Wash the glass slide 2 times with PBS.
- 13) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry.
- 14) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; A549)

## RELATED PRODUCTS:

PM062 Anti-EEA1 pAb  
M176-3 Anti-EEA1 mAb (3C10)  
M176-A48 Anti-EEA1 mAb-Alexa Fluor<sup>®</sup> 488 (3C10)  
M176-A59 Anti-EEA1 mAb-Alexa Fluor<sup>®</sup> 594 (3C10)  
M176-A64 Anti-EEA1 mAb-Alexa Fluor<sup>®</sup> 647 (3C10)  
M175-3 Anti- $\alpha$ -Tubulin mAb (2F9)  
M175-A48 Anti- $\alpha$ -Tubulin mAb-Alexa Fluor<sup>®</sup> 488 (2F9)  
M175-A59 Anti- $\alpha$ -Tubulin mAb-Alexa Fluor<sup>®</sup> 594 (2F9)  
M175-A64 Anti- $\alpha$ -Tubulin mAb-Alexa Fluor<sup>®</sup> 647 (2F9)  
PM054 Anti- $\alpha$ -Tubulin pAb  
PM054-7 Anti- $\alpha$ -Tubulin pAb-HRP-Direct  
M178-3 Anti-Calnexin mAb (4F10)  
M178-A48 Anti-Calnexin mAb-Alexa Fluor<sup>®</sup> 488 (4F10)  
M178-A59 Anti-Calnexin mAb-Alexa Fluor<sup>®</sup> 594 (4F10)  
M178-A64 Anti-Calnexin mAb-Alexa Fluor<sup>®</sup> 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<sup>®</sup> 488 (5G8)  
M179-A59 Anti-GM130 mAb-Alexa Fluor<sup>®</sup> 594 (5G8)  
M179-A64 Anti-GM130 mAb-Alexa Fluor<sup>®</sup> 647 (5G8)  
PM061 Anti-GM130 pAb  
PM063 Anti-COX4 pAb  
PM064 Anti-Lamin B1 pAb

D115-3 Anti-CENP-A (Human) mAb (3-19)  
PD030 Anti-CENP-C (Human) pAb  
K0171-3 Anti-CENP-E (Human) mAb (1H12)  
PD031 Anti-CENP-H (Human) pAb  
PD032 Anti-CENP-I (hMis6) (Human) pAb  
D282-3 Anti-CENP-K (ICEN37) (Human) mAb (46F1)  
PD018 Anti-CENP-K (ICEN37) (Human) pAb  
D283-3 Anti-CENP-L (ICEN33) (Human) mAb (27E10)  
D284-3 Anti-CENP-M (ICEN39) (Human) mAb (23F6)  
D285-3 Anti-CENP-N (ICEN32) (Human) mAb (22F4)  
PD020 Anti-CENP-O (Chicken) pAb  
D286-3 Anti-CENP-T (ICEN22) (Human) mAb (42F10)  
PD019 Anti-CENP-50 (Human) pAb  
D288-3 Anti-MgcRacGAP (Human) mAb (5G5)

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]  
PM040 Anti-Atg16L pAb  
M150-3 Anti-Atg16L mAb (1F12)

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/>