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Not for use in diagnostic procedures.

MBL
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POLYCLONAL ANTIBODY **Early Endosome Marker**

Anti-EEA1 pAb

Code No.	Quantity	Form
PM062	100 µL	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 Antigen 1) 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: 100 µL 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°C.

REACTIVITY: This antibody reacts with EEA1 for Western blotting, Immunoprecipitation and Immunocytochemistry.

APPLICATIONS:
Western blotting; 1:1,000
Immunoprecipitation; 2 µL/300 µL of cell extract from 1 x 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:
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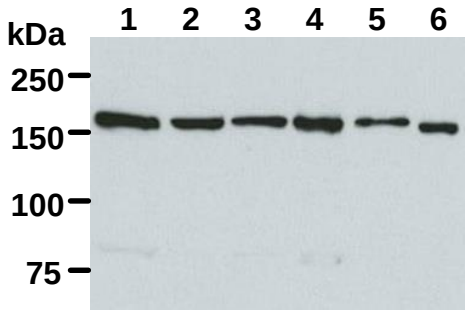
REFERENCES:
1) Abe, S., *et al*., *J. Biol. Chem.* **292**, 4089-4098 (2017) [IC]
2) Gaullier, J. M., *et al., J. Biol. Chem.* **275**, 24595-24600 (2000)
3) Mu, F. T., *et al., J. Biol. Chem*. **270**, 13503-13511 (1995)

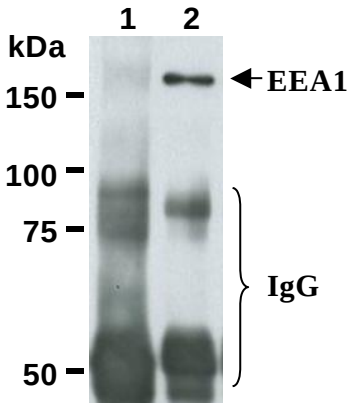


Western blotting analysis of EEA1 expression in HeLa (1), 293T (2), A549 (3), NIH/3T3 (4), MEF (5) and NRK (6) using PM062.

PROTOCOLS:
SDS-PAGE & Western blotting
1) Wash cells (approximately 1 x 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 µL 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^2 for 1 hour 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.
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°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).
7) Incubate the membrane with 1:10,000 Anti-IgG (Rabbit) pAb-HRP (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).
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

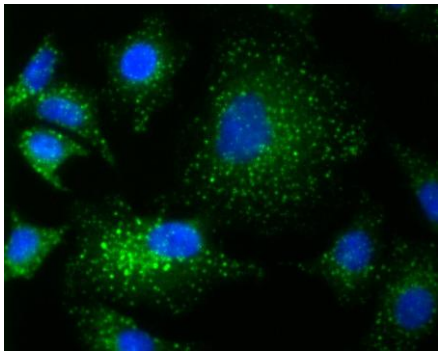
1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 1 mL of cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] containing protease inhibitors at appropriate concentrations. Incubate it at 4°C with rotating for 30 minutes; thereafter, briefly sonicate 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 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL 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) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
5) Resuspend the agarose with cold Lysis buffer.
6) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
7) Repeat steps 5)-6) 2-4 times.
8) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; HeLa)

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 1 x 10^4 cells for one slide, then incubate in a CO_2 incubator overnight.)
2) Wash the glass slide twice 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 twice 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 twice with PBS.
9) Add 100 µL of 1:500 Alexa Fluor® 488 conjugated anti-rabbit IgG (Thermo Fisher Scientific, code no. A11008) 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) Counterstain with DAPI for 5 minutes at room temperature.
12) Wash the glass slide twice 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)



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

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