Anti-EEA1 Antibody [JE59-34]

HA722147

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Monkey
Applications:	WB, IF-Cell, FC
Molecular Wt:	Predicted band size: 162 kDa
Clone number:	JE59-34

Description: The gene EEA1 encodes for the 1400 amino acid protein, Early Endosome Antigen 1. EEA1 localizes exclusively to early endosomes and has an important role in endosomal trafficking. EEA1 binds directly to the phospholipid phosphatidylinositol 3-phosphate through its C-terminal FYVE domain and forms a homodimer through a coiled coil. EEA1 acts as a tethering molecule that couples vesicle docking with SNAREs such as N-ethylmaleimide sensitive fusion protein, bringing the endosomes physically closer and ultimately resulting in the fusion and delivery of endosomal cargo. EEA1 is a RAB5A effector protein which binds via an N-terminal zinc finger domain and is required for fusion of early and late endosomes and for sorting at the early endosome level. EEA1 plays a role in endocytosis and is recruited by Rab5-GTP to endosomal membranes. EEA1 may be regulated through monoubiquination, affecting endosome fusion and trafficking. Ubiquitin selective segregase p97 may regulate EEA1's tethering ability, affecting its endosome trafficking and morphplogy.

Immunogen: Recombinant protein within Human EEA1 aa 1-100 / 1,411.

Positive control: HeLa cell lysate, Jurkat cell lysate, JAR cell lysate, NIH/3T3 cell lysate, C6 cell lysate, COS-1 cell lysate, JAR, NIH/3T3, C6.

Subcellular location: Cytoplasm, Early endosome membrane.

Database links: SwissProt: Q15075 Human | Q8BL66 Mouse
Entrez Gene: 314764 Rat

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:100
FC	1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

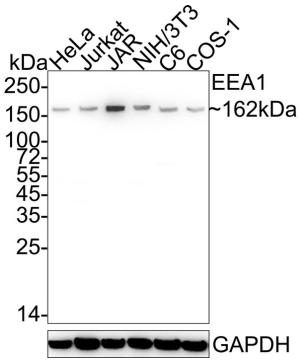

Fig1: Western blot analysis of EEA1 on different lysates with Rabbit anti-EEA1 antibody (HA722147) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: Jurkat cell lysate
Lane 3: JAR cell lysate
Lane 4: NIH/3T3 cell lysate
Lane 5: C6 cell lysate
Lane 6: COS-1 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 162 kDa
Observed band size: 162 kDa

Exposure time: 12 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA722147) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

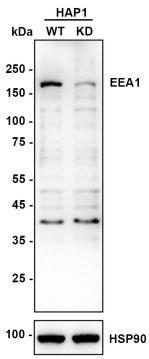

Fig2: Western blot analysis of EEA1 on different lysates with Rabbit anti-EEA1 antibody (HA722147) at 1/1,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-EEA1 KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 162 kDa
Observed band size: 162 kDa

Exposure time: 120 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA722147) at 1/1,000 dilution was used in K1803 at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

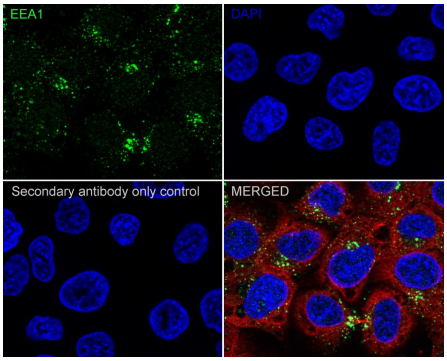

Fig3: Immunocytochemistry analysis of JAR cells labeling EEA1 with Rabbit anti-EEA1 antibody (HA722147) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-EEA1 antibody (HA722147) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

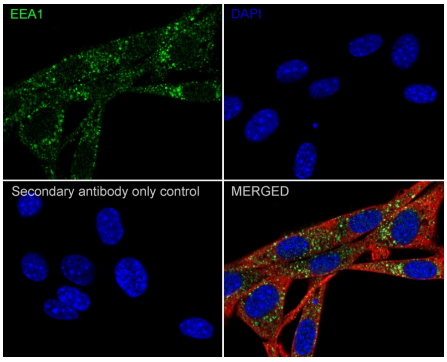

Fig4: Immunocytochemistry analysis of NIH/3T3 cells labeling EEA1 with Rabbit anti-EEA1 antibody (HA722147) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-EEA1 antibody (HA722147) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

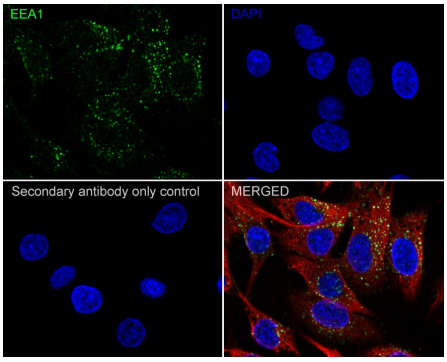

Fig5: Immunocytochemistry analysis of C6 cells labeling EEA1 with Rabbit anti-EEA1 antibody (HA722147) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-EEA1 antibody (HA722147) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

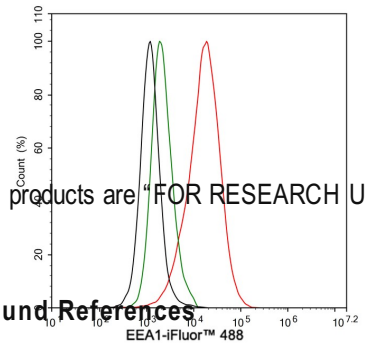

Fig6: Flow cytometric analysis of JAR cells labeling EEA1.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722147, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Kamentseva R et al. Functional cycle of EEA1-positive early endosome: Direct evidence for pre-existing compartment of degradative pathway. PLoS One. 2020 May
2. Larsen AH et al. Membrane-binding mechanism of the EEA1 FYVE domain revealed by multi-scale molecular dynamics simulations. PLoS Comput Biol. 2021 Sep