abcam

Product datasheet

Anti-EEA1 antibody [EPR4245] - Early Endosome Marker ab109110

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-EEA1 antibody [EPR4245] - Early Endosome Marker
Description	Rabbit monoclonal [EPR4245] to EEA1 - Early Endosome Marker
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, WB **Unsuitable for:** IP
Species reactivity	**Reacts with:** Mouse, Rat, Human, African green monkey
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: COS-1, NIH 3T3, C6, HeLa, Jurkat, Daudi, SH-SY5Y and JAR cell lysates. ICC/IF: JAR cells.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 9% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA, 50% Tissue culture supernatant
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR4245
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab109110 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF	★★★★★ (1)	1/500 - 1/1000.
WB	★★★★★ (2)	1/10000 - 1/50000. Detects a band of approximately 170 kDa (predicted molecular weight: 162 kDa).

Application notes Is unsuitable for IP.

Target

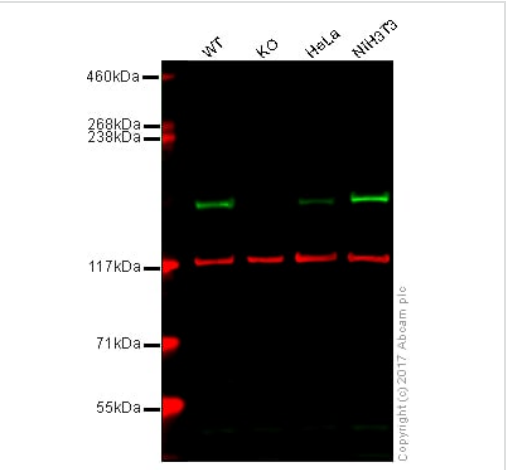
Function Binds phospholipid vesicles containing phosphatidylinositol 3-phosphate and participates in endosomal trafficking.

Sequence similarities Contains 1 C2H2-type zinc finger.
Contains 1 FYVE-type zinc finger.

Domain The FYVE-type zinc finger domain mediates interactions with phosphatidylinositol 3-phosphate in membranes of early endosomes and penetrates bilayers. The FYVE domain insertion into PtdIns(3)P-enriched membranes is substantially increased in acidic conditions.

Cellular localization Cytoplasm. Early endosome membrane.

Images



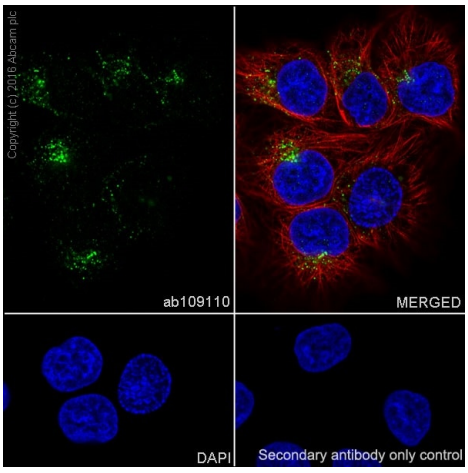
Western blot - Anti-EEA1 antibody [EPR4245] - Early Endosome Marker (ab109110)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)
Lane 2: Early Endosome Marker knockout HAP1 whole cell lysate (20 µg)
Lane 3: HeLa whole cell lysate (20 µg)
Lane 4: NIH3T3 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab109110 observed at 162 kDa. Red - loading control, **ab18058**, observed at 130 kDa.

ab109110 was shown to recognize Early Endosome Marker in wild-type HAP1 cells as signal was lost at the expected MW in Early Endosome Marker knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and Early Endosome Marker knockout samples were subjected to SDS-PAGE. Ab109110 and **ab18058** (Mouse anti-Vinculin loading

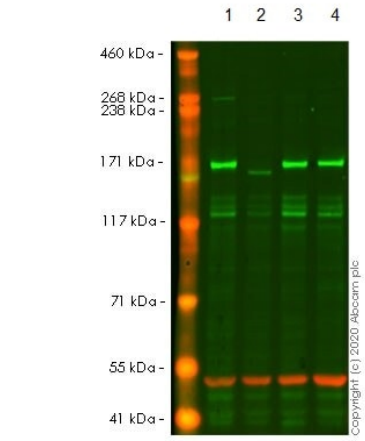
control) were incubated overnight at 4°C at 1/10000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-EEA1 antibody [EPR4245] - Early Endosome Marker (ab109110)

Immunocytochemistry/Immunofluorescence analysis of JAR (human placenta choriocarcinoma epithelial) cells labelling EEA1 with ab109110 at a dilution of 1/250. Cells were fixed with 4% paraformaldehye and permeabilized with 0.1% TritonX-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG was used as the secondary antibody at a dilution of 1/1000. Counterstained with DAPI and **ab195889**, anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594), at a dilution of 1/200.

Image shows cytoplasmic staining in JAR cell line.



Western blot - Anti-EEA1 antibody [EPR4245] - Early Endosome Marker (ab109110)

All lanes : Anti-EEA1 antibody [EPR4245] - Early Endosome Marker (ab109110) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : EEA1 CRISPR/Cas9 edited HeLa cell lysate
Lane 3 : Daudi cell lysate
Lane 4 : SH-SY5Y cell lysate

Lysates/proteins at 20 µg per lane.

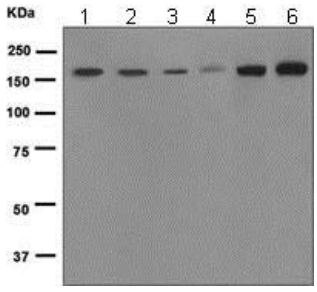
Performed under reducing conditions.

Predicted band size: 162 kDa
Observed band size: 175 kDa

Lanes 1- 4: Merged signal (red and green). Green - ab109110 observed at 175 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab109110 was shown to react with EEA1 in wild-type HeLa cells in

western blot. The band observed in CRISPR/Cas9 edited cell line **ab261822** (CRISPR/Cas9 edited cell lysate **ab256897**) lane below 175kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HeLa and EEA1 CRISPR/Cas9 edited HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab109110 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 in 10000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-EEA1 antibody [EPR4245] - Early Endosome Marker (ab109110)

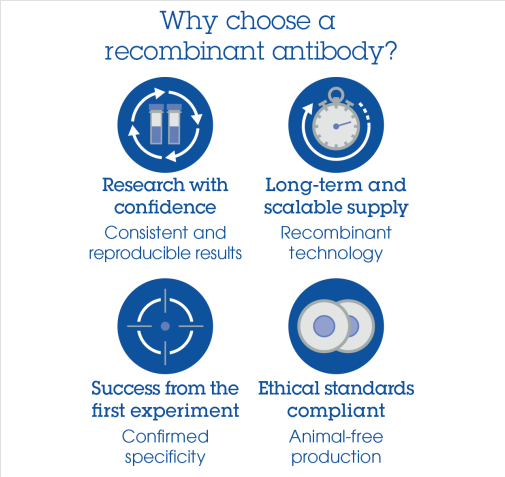
All lanes : Anti-EEA1 antibody [EPR4245] - Early Endosome Marker (ab109110) at 1/10000 dilution

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

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 162 kDa
Observed band size: 170 kDa



Anti-EEA1 antibody [EPR4245] - Early Endosome Marker (ab109110)

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