

# Anti-EEA1 antibody - Early Endosome Marker ab2900


KO 評価済

★★★★☆ 38 Abreviews 239 References 画像数 8

## 製品の概要

製品名	Anti-EEA1 antibody - Early Endosome Marker
製品の詳細	Rabbit polyclonal to EEA1 - Early Endosome Marker
由来種	Rabbit
特異性	Detects a band at 180kDa that represents EEA1 in Western blotting on human cell lines (corresponds to results seen in Mu et al). Also detects a band at 100kDa, we are unsure as to the identity of this band. Immunofluorescence staining of EEA1 in HeLa cells yields a punctate staining pattern consistent with the cytoplasmic distribution of endosomes. From Jan 2024, QC testing of replenishment batches of this polyclonal changed. All tested and expected application and reactive species combinations are still covered by our Abcam product promise. However, we no longer test all applications. For more information on a specific batch, please contact our Scientific Support who will be happy to help. You may also be interested in our alternative recombinant antibody, <a href="#">ab109110</a> .

## アプリケーション

種交差性	適用あり: ICC/IF, WB 交差種: Mouse, Rat, Dog, Human, Xenopus laevis, Chinese hamster 交差が予測される動物種: Chicken, Hamster, Cow, Zebrafish, Rhesus monkey, Aplysia 
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	ICC: HepG2 cells
特記事項	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.  If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

## 製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.

バッファー	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS
	Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.
精製度	Immunogen affinity purified
ポリ/モノ	ポリクローナル
アイソタイプ	IgG

## アプリケーション

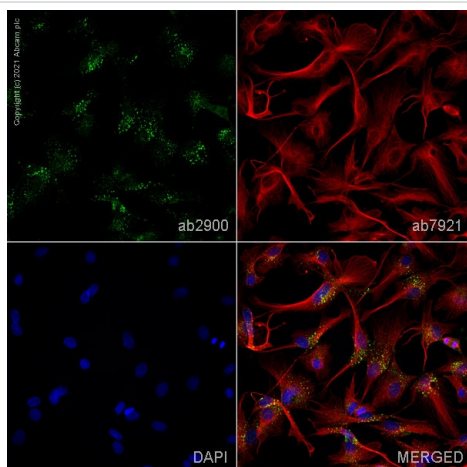
**The Abpromise guarantee**      **Abpromise保証は、次のテスト済みアプリケーションにおけるab2900の使用に適用されます**  
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
ICC/IF	★★★★★ (23)	Use a concentration of 1 - 5 µg/ml.
WB	★★★★★ (12)	Use a concentration of 1 µg/ml. Detects a band of approximately 180 kDa (predicted molecular weight: 160 kDa). Abcam recommends using milk as the blocking agent.

## ターゲット情報

機能	Binds phospholipid vesicles containing phosphatidylinositol 3-phosphate and participates in endosomal trafficking.
配列類似性	Contains 1 C2H2-type zinc finger. Contains 1 FYVE-type zinc finger.
ドメイン	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.
細胞内局在	Cytoplasm. Early endosome membrane.

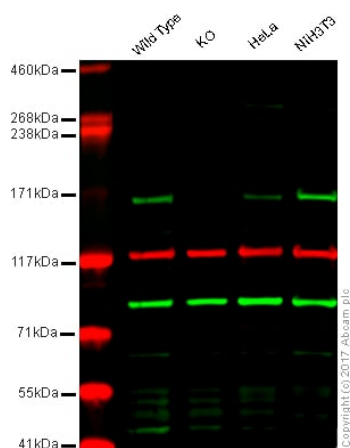
## 画像



Immunocytochemistry/ Immunofluorescence - Anti-EEA1 antibody - Early Endosome Marker (ab2900)

ab2900 staining EEA1 in HepG2 cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab2900 at 5µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Western blot - Anti-EEA1 antibody - Early Endosome Marker (ab2900)

**Lane 1:** Wild-type HAP1 whole cell lysate (20 µg)

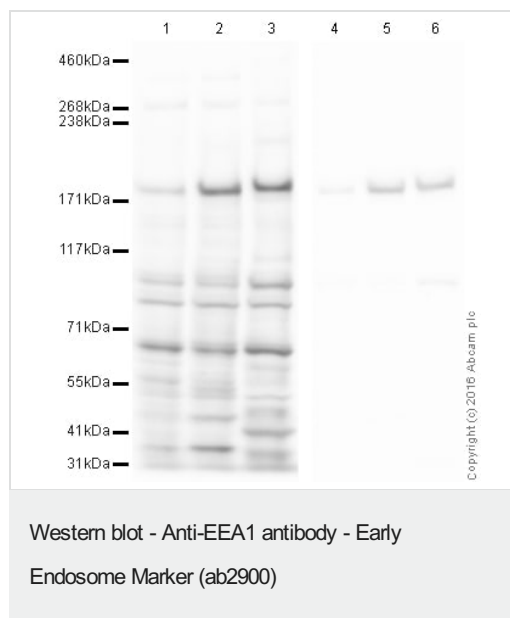
**Lane 2:** EEA1 knockout HAP1 whole cell lysate (20 µg)

**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 - ab2900 observed at 162 kDa. Red - loading control, **ab18058**, observed at 130 kDa.

ab2900 was shown to recognize EEA1 in wild-type HAP1 cells as signal was lost at the expected MW in EEA1 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and EEA1 knockout samples were subjected to SDS-PAGE. Ab2900 and **ab18058** (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1 µg/ml 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.



**All lanes :** Anti-EEA1 antibody - Early Endosome Marker (ab2900)  
at 1 µg/ml

**Lane 1 :** HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

**Lane 2 :** HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

**Lane 3 :** NIH/3T3 (Mouse embryo fibroblast cell line) whole cell lysate

**Lane 4 :** HeLa whole cell lysate

**Lane 5 :** HEK-293 whole cell lysate

**Lane 6 :** NIH 3T3 whole cell lysate

Lysates/proteins at 20 µg per lane.

### Secondary

**All lanes :** Goat polyclonal to Rabbit IgG H&L (HRP) at 1/50000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size:** 160 kDa

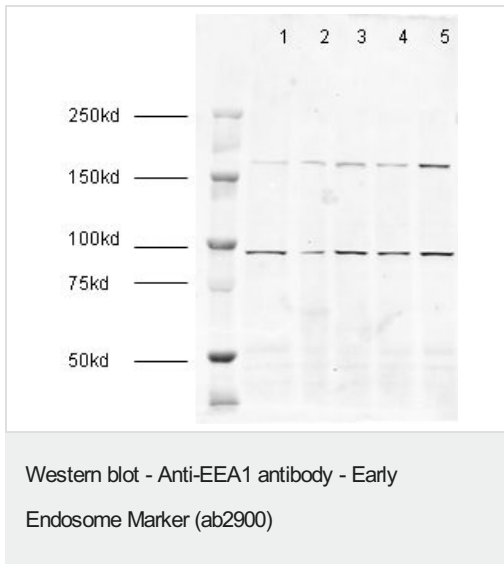
**Observed band size:** 180 kDa

**Additional bands at:** 100 kDa. We are unsure as to the identity of these extra bands.

**Exposure time:** 30 seconds

This blot was produced using a 3-8% Tris Acetate gel under the TA buffer system. The gel was run at 150V for 60 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin (Lanes 1-3) or 3% Milk (Lanes 4-6) before being incubated with ab2900 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution [ab133406](#).

Abcam recommends using milk as the blocking agent. Abcam welcomes customer feedback and would appreciate any comments regarding this product and the data presented above.



**All lanes :** Anti-EEA1 antibody - Early Endosome Marker (ab2900) at 1/1000 dilution

**Lane 1 :** HeLa (Human epithelial cell line from cervix adenocarcinoma) nuclear lysate

**Lane 2 :** HeLa whole cell lysate

**Lane 3 :** A431 (Human epidermoid carcinoma cell line) whole cell lysate

**Lane 4 :** Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

**Lane 5 :** HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

Lysates/proteins at 20 µg per lane.

### Secondary

**All lanes :** Alexa Fluor anti rabbit at 1/50000 dilution

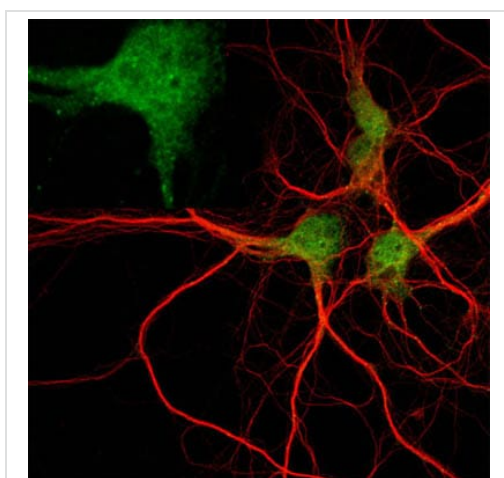
Performed under reducing conditions.

**Predicted band size:** 160 kDa

**Observed band size:** 180 kDa

**Additional bands at:** 100 kDa, 41 kDa, 50 kDa. We are unsure as to the identity of these extra bands.

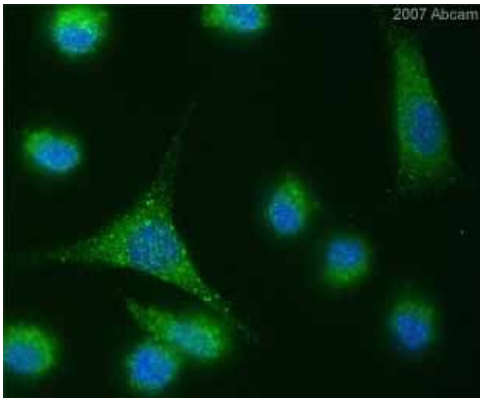
Fluorescence detection of secondary antibody.



Confocal microscopy of fixed primary cultures of rat hippocampal neurons (embryonic day 18) showing immunocytochemical labelling of rabbit polyclonal to EEA1 (ab2900, 1/200; Alexa Fluor 488 1/200; green) and monoclonal mouse anti-β.

Immunocytochemistry/ Immunofluorescence - Anti-EEA1 antibody - Early Endosome Marker (ab2900)

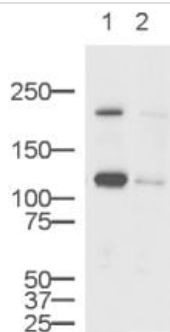
This image is courtesy of Randal Moldrich, CNRS UMR7637, ESPCI, France



Immunocytochemistry/ Immunofluorescence - Anti-EEA1 antibody - Early Endosome Marker (ab2900)

This image is courtesy of an anonymous Abreview

ab2900 staining mouse L-cells by ICC/IF. Cells were formaldehyde fixed, permeabilized in Triton X-100 and incubated with ab2900 diluted 1/1000 for 1 hour at 37°C. A Cy2® conjugated goat anti-rabbit antibody was used as the secondary.



Western blot - Anti-EEA1 antibody - Early Endosome Marker (ab2900)

**All lanes :** Anti-EEA1 antibody - Early Endosome Marker (ab2900) at 1/500 dilution

**Lane 1 :** HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

**Lane 2 :** HEK293 Whole Cell lysate with Human EEA1 peptide ([ab14946](#)) at 1 µg

Lysates/proteins at 20 µg per lane.

### Secondary

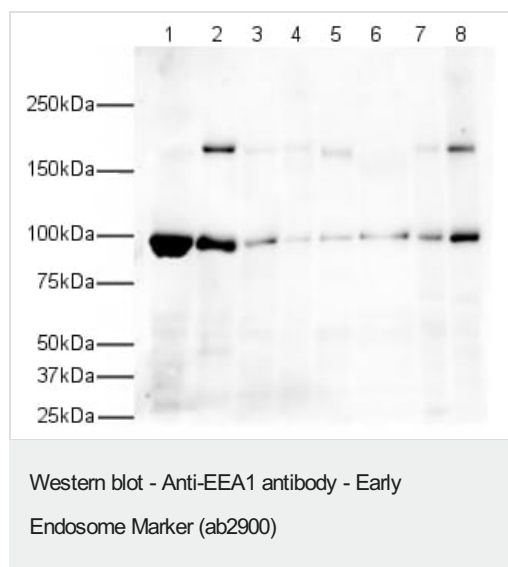
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) ([ab6721](#)) at 1/5000 dilution

Performed under reducing conditions.

**Predicted band size:** 160 kDa

**Observed band size:** 180 kDa

Lane 1 - 2 : EEA1 antibody - Early Endosome Marker (ab2900) at 1/500 dilution, HEK293 Whole Cell lysate at 20 ug Lane 1 : as above Lane 2 : EEA1 peptide ([ab14946](#)) at 1 ug Secondary Goat polyclonal to Rabbit IgG H&L (HRP) ([ab6721](#)) at 1/5000 dilution Performed under reducing conditions. Predicted band size : 160kD Observed band size : 180kD (why is the actual band size different from the predicted?)



**All lanes :** Anti-EEA1 antibody - Early Endosome Marker (ab2900)  
at 1 µg/ml

**Lane 1 :** Xenopus laevis lysate

**Lane 2 :** Mouse 3T3 cell lysate

**Lane 3 :** Mouse brain cell lysate

**Lane 4 :** Mouse liver cell lysate

**Lane 5 :** Rat brain cell lysate

**Lane 6 :** Rat liver cell lysate

**Lane 7 :** Dog lysate

**Lane 8 :** CHO cell lysate

Lysates/proteins at 20 µg per lane.

### Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) ([ab6721](#)) at 1/5000 dilution

Performed under reducing conditions.

**Predicted band size:** 160 kDa

**Observed band size:** 100,180 kDa

**Exposure time:** 30 seconds

The Western blot shows that [ab14944](#) reacts strongly with mouse 3T3 and CHO cell lysates. Weak cross-reactivity is seen with Xenopus, mouse brain, mouse liver, rat brain and dog lysates. The antibody does not appear to cross-react with rat liver lysate.

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