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Anti-EEA1 Antibody Picoband™

Catalog Number: A02296-3

About EEA1

The gene EEA1 encodes for the 1400 amino acid protein, Early Endosome Antigen 1. It 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 ahomodimer through a coiled coil. Furthermore, 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.

Overview

Product Name	Anti-EEA1 Antibody Picoband™
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-EEA1 Antibody Picoband™ catalog # A02296-3. Tested in IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.01mg NaN3.
Storage Instructions	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	Q15075

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human EEA1, identical to the related mouse and rat sequences.
Predicted Reactive Species	Human
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.

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Purification	Immunogen affinity purified.
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples. Some PubMed article(s) citing the expression level of this target are as follows: Boster Bio's internal QC testing used: Western blot, 0.1-0.5ug/ml Immunohistochemistry, 2-5 ug/ml Immunocytochemistry/Immunofluorescence, 5ug/ml

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Anti-EEA1 Antibody Picoband™ (A02296-3) Images

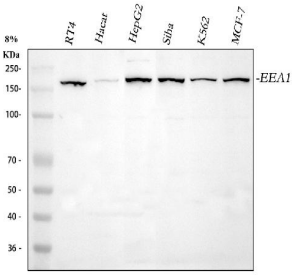

Figure 1. Western blot analysis of EEA1 using anti-EEA1 antibody (A02296-3).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human RT4 whole cell lysates,
Lane 2: human Hacat whole cell lysates,
Lane 3: human HepG2 whole cell lysates,
Lane 4: human SiHa whole cell lysates,
Lane 5: humna K562 whole cell lysates,
Lane 6: humna MCF-7 whole cell lysates.
After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-EEA1 antigen affinity purified polyclonal antibody (Catalog # A02296-3) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for EEA1 at approximately 162 kDa. The expected band size for EEA1 is at 162 kDa.

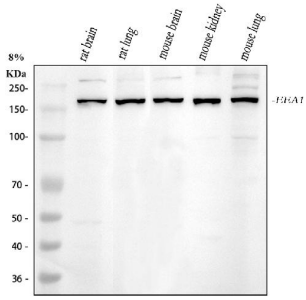

Figure 2. Western blot analysis of EEA1 using anti-EEA1 antibody (A02296-3).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: rat brain tissue lysates,
Lane 2: rat lung tissue lysates,
Lane 3: mouse brain tissue lysates,
Lane 4: mouse kidney tissue lysates,
Lane 5: mouse lung tissue lysates.
After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-EEA1 antigen affinity purified polyclonal antibody (Catalog # A02296-3) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for EEA1 at approximately 162 kDa. The expected band size for EEA1 is at 162 kDa.

Figure 3. IHC analysis of EEA1 using anti-EEA1 antibody (A02296-3).

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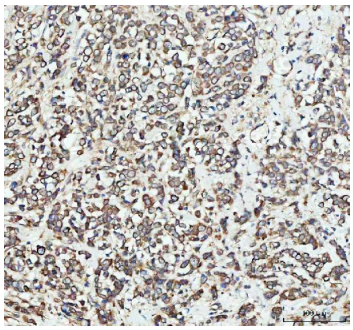

EEA1 was detected in a paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-EEA1 Antibody (A02296-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

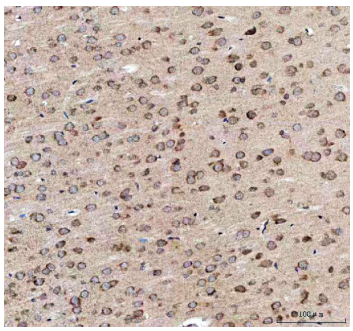

Figure 4. IHC analysis of EEA1 using anti-EEA1 antibody (A02296-3).
EEA1 was detected in a paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-EEA1 Antibody (A02296-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

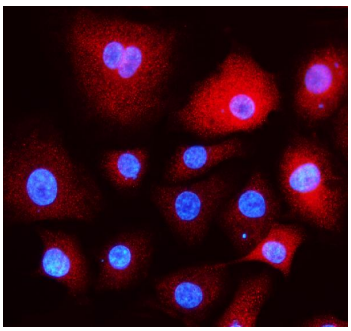

Figure 5. IF analysis of EEA1 using anti- EEA1 antibody (A02296-3).
EEA1 was detected in immunocytochemical section of A549 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5ug/mL rabbit anti- EEA1 Antibody (A02296-3) overnight at 4°C. DyLight®550 Conjugated Goat Anti-Rabbit IgG (BA1135) was used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

1 Publications Citing This Product

1. PubMed ID: -, Wang,Y.,Pang,J.,Wang,Q.,Yan,L.,Wang,L.,Xing,Z.,Wang,C.,Zhang,J., Dong,L.,Delivering Antisense Oligonucleotides across the Blood□Brain Barrier by Tumor Cell□Derived Small Apoptotic Bodies.Adv.Sci.2021,2004929.https://doi.org/10.1002/advs.202004929

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