

## **Anti-CARD4 Picoband Antibody**

Catalog # ABO11985

## **Specification**

# **Anti-CARD4 Picoband Antibody - Product Information**

Application WB, IHC-P, ICC

Primary Accession

Host

Rabbit

Reactivity Human, Mouse, Rat

Clonality Polyclonal Lyophilized

**Description** 

Rabbit IgG polyclonal antibody for Nucleotide-binding oligomerization domain-containing protein 1(NOD1) detection. Tested with WB, IHC-P, ICC in Human; Mouse; Rat.

#### Reconstitution

Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

### **Anti-CARD4 Picoband Antibody - Additional Information**

**Gene ID** 10392

#### **Other Names**

Nucleotide-binding oligomerization domain-containing protein 1, Caspase recruitment domain-containing protein 4, NOD1, CARD4

# Calculated MW 107691 MW KDa

### **Application Details**

Immunohistochemistry(Paraffin-embedded Section), 0.5-1 μg/ml, By Heat<br/>br>Immunocytochemistry, 0.5-1 μg/ml<br/>br>Western blot, 0.1-0.5 μg/ml<br/>br>

# **Subcellular Localization**

Cytoplasm. Cell membrane. Apical cell membrane. Basolateral cell membrane. Detected in the cytoplasm and at the cell membrane. Following bacterial infection, localizes to bacterial entry sites in the cell membrane. Recruited to the basolateral and apical membranes in polarized epithelial cells.

#### **Tissue Specificity**

Highly expressed in adult heart, skeletal muscle, pancreas, spleen and ovary. Also detected in placenta, lung, liver, kidney, thymus, testis, small intestine and colon.

### **Protein Name**

Nucleotide-binding oligomerization domain-containing protein 1

#### Contents

Each vial contains 5mg BSA, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.



## **Immunogen**

E.coli-derived human CARD4 recombinant protein (Position: M1-M160). Human CARD4 shares 82% amino acid (aa) sequence identity with mouse CARD4.

#### **Purification**

Immunogen affinity purified.

## **Cross Reactivity**

No cross reactivity with other proteins

Storage

At -20°C for one year. After r°Constitution, at 4°C for one month. It°Can also be aliquotted and stored frozen at -20°C for a longer time. Avoid repeated freezing and thawing.

**Sequence Similarities**Contains 1 CARD domain.

# **Anti-CARD4 Picoband Antibody - Protein Information**

Name NOD1 {ECO:0000303|PubMed:10329646, ECO:0000312|HGNC:HGNC:16390}

#### **Function**

Pattern recognition receptor (PRR) that detects bacterial peptidoglycan fragments and other danger signals and thus participates in both innate and adaptive immune responses (PubMed: <a href="http://www.uniprot.org/citations/11058605" target="\_blank">11058605</a>, PubMed:<a href="http://www.uniprot.org/citations/12791997" target="blank">12791997</a>, PubMed:<a href="http://www.uniprot.org/citations/12796777" target="\_blank">12796777</a>, PubMed:<a href="http://www.uniprot.org/citations/15044951" target="blank">15044951</a>, PubMed:<a href="http://www.uniprot.org/citations/16172124" target="blank">16172124</a>, PubMed:<a href="http://www.uniprot.org/citations/19043560" target="blank">19043560</a>, PubMed:<a href="http://www.uniprot.org/citations/22672233" target="blank">22672233</a>, PubMed:<a href="http://www.uniprot.org/citations/27099311" target="blank">27099311</a>). Specifically recognizes and binds gamma-D-glutamyl-meso-diaminopimelic acid (iE- DAP), a dipeptide present in peptidoglycan of Gram-negative bacteria (PubMed:<a href="http://www.uniprot.org/citations/12791997" target="\_blank">12791997</a>, PubMed:<a href="http://www.uniprot.org/citations/12796777" target="\_blank">12796777</a>, PubMed:<a href="http://www.uniprot.org/citations/12871942" target="blank">12871942</a>, PubMed:<a href="http://www.uniprot.org/citations/16172124" target="\_blank">16172124</a>, PubMed:<a href="http://www.uniprot.org/citations/16211083" target="blank">16211083</a>). Preferentially binds iE-DAP in tripeptide-containing muropeptides (MurNAc-TriDAP or TriDAP) (PubMed: <a href="http://www.uniprot.org/citations/16211083" target="\_blank">16211083</a>). Ligand binding triggers oligomerization that facilitates the binding and subsequent activation of the proximal adapter receptor-interacting RIPK2 (PubMed:<a href="http://www.uniprot.org/citations/12791997" target=" blank">12791997</a>, PubMed:<a href="http://www.uniprot.org/citations/12796777" target="blank">12796777</a>, PubMed:<a href="http://www.uniprot.org/citations/17054981" target="blank">17054981</a>). Following recruitment, RIPK2 undergoes 'Met-1'- (linear) and 'Lys-63'-linked polyubiquitination by E3 ubiquitin-protein ligases XIAP, BIRC2, BIRC3 and the LUBAC complex, becoming a scaffolding protein for downstream effectors, triggering activation of the NF-kappa-B and MAP kinases signaling (PubMed:<a href="http://www.uniprot.org/citations/10880512" target=" blank">10880512</a>, PubMed:<a href="http://www.uniprot.org/citations/12791997" target="blank">12791997</a>, PubMed:<a href="http://www.uniprot.org/citations/19043560" target="blank">19043560</a>). This in turn leads to the transcriptional activation of hundreds of genes involved in immune response (PubMed:<a



href="http://www.uniprot.org/citations/10880512" target="\_blank">10880512</a>, PubMed:<a href="http://www.uniprot.org/citations/19043560" target="\_blank">19043560</a>). Also acts as a regulator of antiviral response elicited by dsRNA and the expression of RLR pathway members by targeting IFIH1 and TRAF3 to modulate the formation of IFIH1-MAVS and TRAF3-MAVS complexes leading to increased transcription of type I IFNs (PubMed:<a

href="http://www.uniprot.org/citations/32169843" target="\_blank">32169843</a>). Also acts as a regulator of autophagy via its interaction with ATG16L1, possibly by recruiting ATG16L1 at the site of bacterial entry (By similarity). Besides recognizing pathogens, also involved in the endoplasmic reticulum stress response: acts by sensing and binding to the cytosolic metabolite sphingosine-1-phosphate generated in response to endoplasmic reticulum stress, initiating an inflammation process that leads to activation of the NF-kappa-B and MAP kinases signaling (PubMed:<a href="http://www.uniprot.org/citations/27007849" target="\_blank">27007849</a>, PubMed:<a href="http://www.uniprot.org/citations/33942347" target="\_blank">33942347</a>). In addition, plays a role in insulin trafficking in beta cells in a cell-autonomous manner (By similarity). Mechanistically, upon recognizing cognate ligands, NOD1 and RIPK2 localize to insulin vesicles where they recruit RAB1A to direct insulin trafficking through the cytoplasm (By similarity).

#### **Cellular Location**

Cell membrane; Lipid-anchor. Apical cell membrane. Basolateral cell membrane. Cytoplasm. Note=Detected in the cytoplasm and at the cell membrane (PubMed:31649195). Following bacterial infection, localizes to bacterial entry sites in the cell membrane (PubMed:31649195). Recruited to the basolateral and apical membranes in polarized epithelial cells (PubMed:19043560)

#### **Tissue Location**

Highly expressed in adult heart, skeletal muscle, pancreas, spleen and ovary (PubMed:10224040). Also detected in placenta, lung, liver, kidney, thymus, testis, small intestine and colon (PubMed:10224040).

# **Anti-CARD4 Picoband Antibody - Protocols**

Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- <u>Immunofluorescence</u>
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

### Anti-CARD4 Picoband Antibody - Images



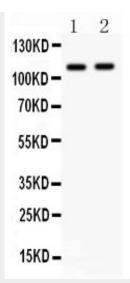


Figure 1. Western blot analysis of CARD4 using anti-CARD4 antibody (ABO11985). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions. Lane 1: A549 Whole Cell Lysate,Lane 2: Rat Cardiac Muscle Tissue Lysate. After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA 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-CARD4 antigen affinity purified polyclonal antibody (Catalog # ABO11985) at 0.5  $1^{1}$ /4g/mL overnight at 4ŰC, 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 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit with Tanon 5200 system. A specific band was detected for CARD4 at approximately 107KD. The expected band size for CARD4 is at 107KD.

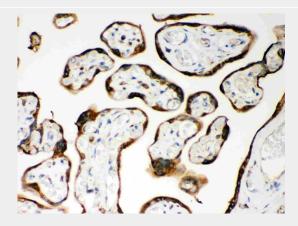


Figure 2. IHC analysis of CARD4 using anti-CARD4 antibody (ABO11985). CARD4 was detected in paraffin-embedded section of Human Placenta Tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with  $1i\frac{1}{4}g/ml$  rabbit anti-CARD4 Antibody (ABO11985) overnight at  $4\hat{A}^{\circ}$ C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at  $37\hat{A}^{\circ}$ C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) with DAB as the chromogen.



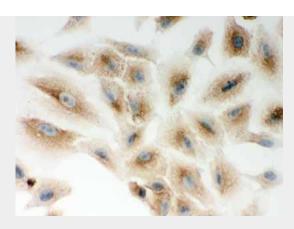


Figure 3. IHC analysis of CARD4 using anti-CARD4 antibody (ABO11985). CARD4 was detected in immunocytochemical section of A549 cell. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with  $11\frac{1}{4}$ g/ml rabbit anti-CARD4 Antibody (ABO11985) overnight at 44°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 374°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) with DAB as the chromogen.

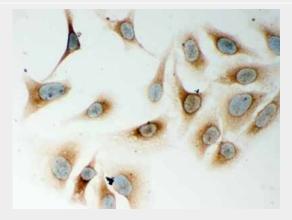


Figure 4. IHC analysis of CARD4 using anti-CARD4 antibody (ABO11985). CARD4 was detected in immunocytochemical section of Hela cell. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with  $11\frac{1}{4}$ g/ml rabbit anti-CARD4 Antibody (ABO11985) overnight at 44°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 374°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) with DAB as the chromogen.

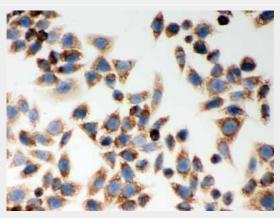


Figure 5. IHC analysis of CARD4 using anti-CARD4 antibody (ABO11985).CARD4 was detected in





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immunocytochemical section of SMMC-7721 cell. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1μg/ml rabbit anti-CARD4 Antibody (ABO11985) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37ŰC. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) with DAB as the chromogen.

# Anti-CARD4 Picoband Antibody - Background

Nucleotide-binding oligomerization domain-containing protein 1, also known as CARD4, is a protein receptor that in humans is encoded by the NOD1 gene. NOD1 is a member of NOD-like receptor protein family and is a close relative of NOD2. NOD1 is mapped to 7p14.3. It recognizes bacterial molecules and stimulates an immune reaction. NOD1 protein contains a caspase recruitment domain (CARD). This gene is an intracellular pattern recognition receptor, which is similar in structure to resistant proteins of plants, and mediates innate and acquired immunity by recognizing bacterial molecules containing D-glutamyl-meso-diaminopimelic acid (iE-DAP) moiety. What wore, it has been shown that NOD1 can sense cytosolic microbial products by monitoring the activation state of small Rho GTPases.