

## Anti-ASC/TMS1/Pycard Antibody Picoband™

Catalog Number: A00362-5

### About Pycard

PYCARD, often referred to as ASC (Apoptosis-associated speck-like protein containing a CARD), is a protein that in humans is encoded by the PYCARD gene. This gene encodes an adaptor protein that is composed of two protein-protein interaction domains: a N-terminal PYRIN-PAAD-DAPIN domain (PYD) and a C-terminal caspase-recruitment domain (CARD). The PYD and CARD domains are members of the six-helix bundle death domain-fold superfamily that mediates assembly of large signaling complexes in the inflammatory and apoptotic signaling pathways via the activation of caspase. In normal cells, this protein is localized to the cytoplasm; however, in cells undergoing apoptosis, it forms ball-like aggregates near the nuclear periphery. Two transcript variants encoding different isoforms have been found for this gene.

### Overview

Product Name	Anti-ASC/TMS1/Pycard Antibody Picoband™
Reactive Species	Mouse
Description	Boster Bio Anti-ASC/TMS1/Pycard Antibody Picoband™ catalog # A00362-5. Tested in ELISA, Flow Cytometry, IF, ICC, WB applications. This antibody reacts with Mouse.
Application	ELISA, Flow Cytometry, IF, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl, 0.2 mg Na <sub>2</sub> HPO <sub>4</sub> .
Storage Instructions	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 freezing and thawing.
Host	Rabbit
Uniprot ID	Q9EPB4

### Technical Details

Immunogen	E.coli-derived mouse ASC/TMS1/Pycard recombinant protein (Position: K24-S193).
Predicted Reactive Species	Chicken
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 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 µg/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	<p>Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.</p> <p>If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.</p> <p>Some PubMed article(s) citing the expression level of this target are as follows:</p> <p>Boster Bio's internal QC testing used:</p> <p>Western blot, 0.25-0.5 µg/ml, Mouse</p> <p>Immunocytochemistry/Immunofluorescence, 5 µg/ml, Mouse</p> <p>Flow Cytometry, 1-3 µg/1x1x10<sup>6</sup> cells, Mouse</p> <p>Direct ELISA, 0.1-0.5 µg/ml, Mouse</p>

## Anti-ASC/TMS1/Pycard Antibody Picoband™ (A00362-5) Images

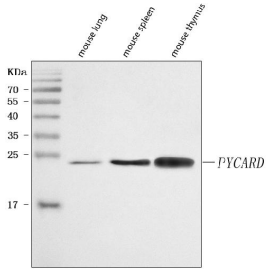


Figure 1. Western blot analysis of ASC/TMS1/Pycard using anti-ASC/TMS1/Pycard antibody (A00362-5). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: mouse lung tissue lysates,  
Lane 2: mouse spleen tissue lysates,  
Lane 3: mouse thymus 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-ASC/TMS1/Pycard antigen affinity purified polyclonal antibody (Catalog # A00362-5) 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 ASC/TMS1/Pycard at approximately 22 kDa. The expected band size for ASC/TMS1/Pycard is at 22 kDa.

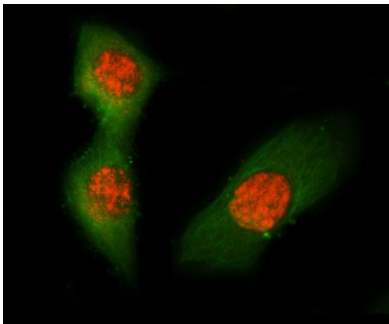


Figure 2. IF analysis of ASC/TMS1/Pycard using anti-ASC/TMS1/Pycard antibody (A00362-5) and anti-Tubulin Alpha antibody (M03989-3).

ASC/TMS1/Pycard was detected in immunocytochemical section of RM1 cell. 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 5 ug/mL rabbit anti-ASC/TMS1/Pycard Antibody (A00362-5) and mouse anti-Tubulin Alpha antibody (M03989-3) overnight at 4°C. Cy3 Conjugated Goat Anti-Rabbit IgG (BA1032) and DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) were used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

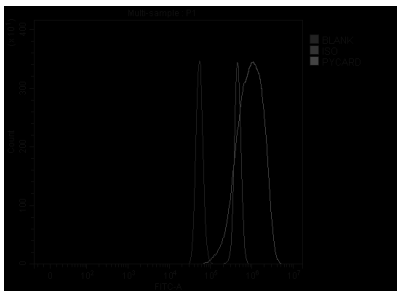


Figure 3. Flow Cytometry analysis of RAW264.7 cells using anti-ASC/TMS1/Pycard antibody (A00362-5).

Overlay histogram showing RAW264.7 cells stained with A00362-5 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-ASC/TMS1/Pycard Antibody (A00362-5, 1 ug/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample (Red line) was also used

as a control.

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