Anti-CARD9 Antibody [PSH0-41] - BSA and Azide free

HA750619

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, FC
Molecular Wt:	Predicted band size: 62.2 kDa
Clone number:	PSH0-41

Description: Caspase recruitment domain-containing protein 9 is an adaptor protein of the CARD-CC protein family, which in humans is encoded by the CARD9 gene. It mediates signals from pattern recognition receptors to activate pro-inflammatory and anti-inflammatory cytokines, regulating inflammation. Homozygous mutations in CARD9 are associated with defective innate immunity against yeasts, like Candida and dermatophytes. CARD9 is a member of the CARD protein family, which is defined by the presence of a characteristic caspase-associated recruitment domain (CARD). This protein was identified by its selective association with the CARD domain of BCL10, a positive regulator and NF-κB activation. It is thought to function as a molecular scaffold for the assembly of a BCL10 signaling complex that activates NF-κB. Several alternatively spliced transcript variants have been observed, but their full-length nature is not clearly defined.

Immunogen: Recombinant protein within human CARD9 aa 1-200 / 536.

Positive control: HL-60 cell lysate, THP-1 cell lysate, RAW264.7 cell lysate, PC-12 cell lysate, THP-1, RAW264.7, PC-12.

Subcellular location: Cytoplasm..

Database links: SwissProt: Q9H257 Human | A2AIV8 Mouse | A2AIV8 Rat

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:100
FC	1:500-1:1,000

Storage Buffer: 1*PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

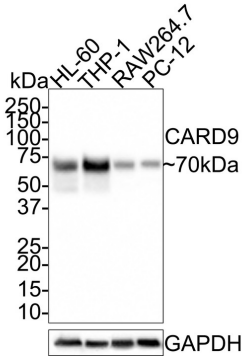

Fig1: Western blot analysis of CARD9 on different lysates with Rabbit anti-CARD9 antibody (HA750619) at 1/1,000 dilution.

Lane 1: HL-60 cell lysate
Lane 2: THP-1 cell lysate
Lane 3: RAW264.7 cell lysate
Lane 4: PC-12 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 62.2 kDa
Observed band size: 70 kDa

Exposure time: 24 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750619) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

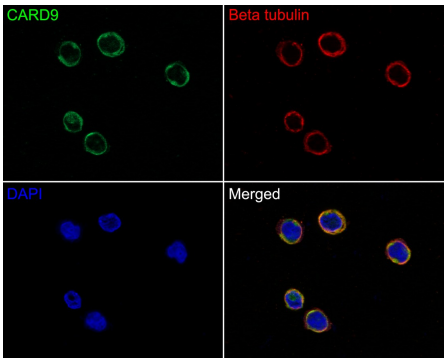

Fig2: Immunocytochemistry analysis of THP-1 cells labeling CARD9 with Rabbit anti-CARD9 antibody (HA750619) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-CARD9 antibody (HA750619) at 1/100 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

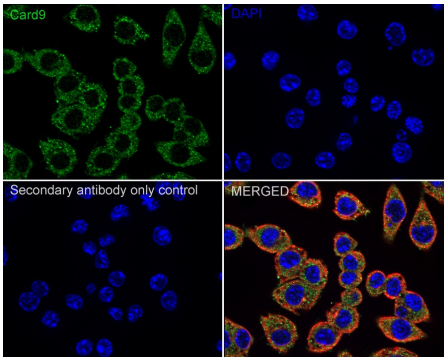

Fig3: Immunocytochemistry analysis of RAW264.7 cells labeling CARD9 with Rabbit anti-CARD9 antibody (HA750619) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-CARD9 antibody (HA750619) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

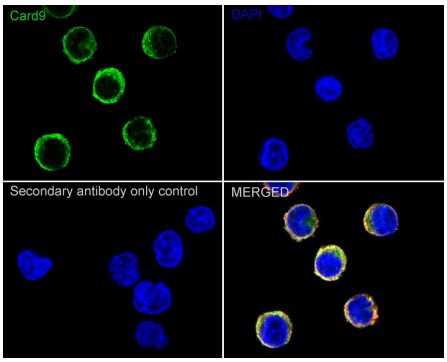

Fig4: Immunocytochemistry analysis of PC-12 cells labeling CARD9 with Rabbit anti-CARD9 antibody (HA750619) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-CARD9 antibody (HA750619) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

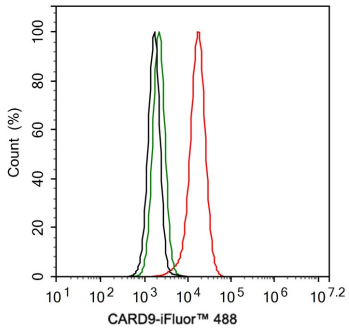

Fig5: Flow cytometric analysis of THP-1 cells labeling CARD9.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750619, 1ug/ml) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

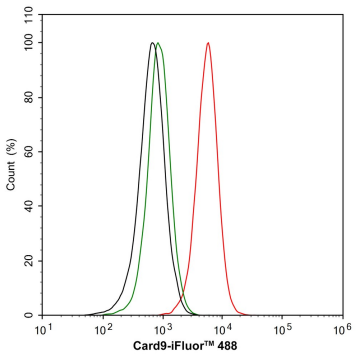

Fig6: Flow cytometric analysis of RAW264.7 cells labeling CARD9.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750619, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Liu X et al. CARD9 Signaling, Inflammation, and Diseases. Front Immunol. 2022 Mar
2. Zhang H et al. CARD9 Regulation and its Role in Cardiovascular Diseases. Int J Biol Sci. 2022 Jan