

Anti-Caspase-9/CASP9 Antibody Picoband™

Catalog Number: A00080-5

About CASP9

CASP9 is also known as MCH6 or APAF3. This gene encodes a member of the cysteine-aspartic acid protease (caspase) family. Sequential activation of caspases plays a central role in the execution-phase of cell apoptosis. Caspases exist as inactive proenzymes which undergo proteolytic processing at conserved aspartic residues to produce two subunits, large and small, that dimerize to form the active enzyme. This protein can undergo autoproteolytic processing and activation by the apoptosome, a protein complex of cytochrome c and the apoptotic peptidase activating factor 1; this step is thought to be one of the earliest in the caspase activation cascade. This protein is thought to play a central role in apoptosis and to be a tumor suppressor. Alternative splicing results in multiple transcript variants.

Overview

Product Name	Anti-Caspase-9/CASP9 Antibody Picoband™
Reactive Species	Human
Description	Boster Bio Anti-Caspase-9/CASP9 Antibody Picoband™ catalog # A00080-5. Tested in ELISA, Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
Application	ELISA, Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na ₂ HPO ₄ .
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	P55211

Technical Details

Immunogen	E.coli-derived human Caspase-9/CASP9 recombinant protein (Position: E3-K410).
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 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.
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, 0.25-0.5ug/ml, Human Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Human Immunocytochemistry/Immunofluorescence, 5ug/ml, Human Flow Cytometry, 1-3ug/1x10⁶ cells, Human Direct ELISA, 0.1-0.5ug/ml, Human

Anti-Caspase-9/CASP9 Antibody Picoband™ (A00080-5) Images

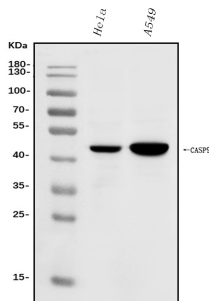


Figure 1. Western blot analysis of Caspase-9/CASP9 using anti-Caspase-9/CASP9 antibody (A00080-5). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30ug of sample under reducing conditions.

Lane 1: human HeLa whole cell lysates,
Lane 2: human A549 whole cell lysates.

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-Caspase-9/CASP9 antigen affinity purified polyclonal antibody (Catalog # A00080-5) 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 Caspase-9/CASP9 at approximately 46KD. The expected band size for Caspase-9/CASP9 is at 46KD.

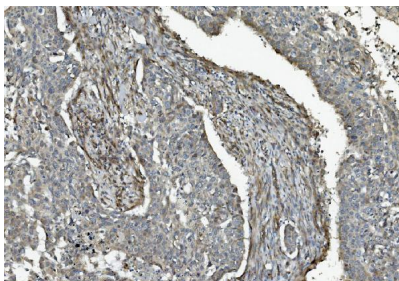


Figure 2. IHC analysis of Caspase-9/CASP9 using anti-Caspase-9/CASP9 antibody (A00080-5). Caspase-9/CASP9 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-Caspase-9/CASP9 Antibody (A00080-5) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

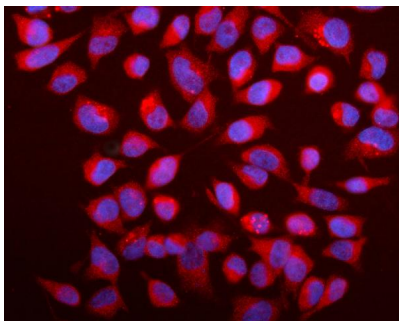
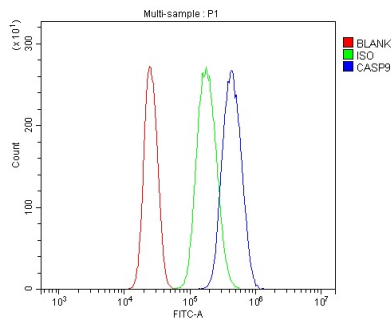


Figure 3. IF analysis of Caspase-9/CASP9 using anti-Caspase-9/CASP9 antibody (A00080-5). Caspase-9/CASP9 was detected in immunocytochemical section of HELA cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5ug/mL rabbit anti-Caspase-9/CASP9 Antibody (A00080-5) overnight at 4°C. DyLight®594 Conjugated Goat Anti-Rabbit IgG (BA1142) was used as secondary antibody at 1:100 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.

Figure 4. Flow Cytometry analysis of U937 cells using anti-



Caspase-9/CASP9 antibody (A00080-5). Overlay histogram showing U937 cells stained with A00080-5 (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-Caspase-9/CASP9 Antibody (A00080-5, 1 μ g/1 $\times$ 10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 μ g/1 $\times$ 10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 μ g/1 $\times$ 10⁶) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

1 Publications Citing This Product

1. PubMed ID: 32802118, Li QY, Hou CZ, Yang LP, Chu XL, Wang Y, Zhang P, Zhao Y. Study on the Mechanism of Ginseng in the Treatment of Lung Adenocarcinoma Based on Network Pharmacology. Evid Based Complement Alternat Med. 2020 Jul 31;2020:2658795. doi:10.1155/2020/2658795. PMID:32802118

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