

# Anti-Caspase-9/CASP9 Antibody Picoband™

Catalog Number: A00080-6

#### **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 # A00080-6. Tested in ELISA, IHC, WB applications. This antibody reacts with Human.
Application	ELISA, Flow Cytometry, IHC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4.
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 CASP9 recombinant protein (Position: E18-S416).
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).
Cross Reactivity	No cross-reactivity with other proteins
Isotype	Rabbit IgG
Form	Lyophilized





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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  Flow Cytometry, 1-3ug/1x10 <sup>6</sup> cells, Human  Direct ELISA, 0.1-0.5ug/ml, Human



### Anti-Caspase-9/CASP9 Antibody Picoband™ (A00080-6) Images

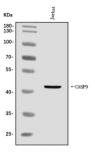


Figure 1. Western blot analysis of Caspase-9/CASP9 using anti-Caspase-9/CASP9 antibody (A00080-6). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: human Jurkat 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-Caspase-9/CASP9 antigen affinity purified polyclonal antibody (Catalog # A00080-6) 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 46 kDa. The expected band size for Caspase-9/CASP9 is at 46 kDa.

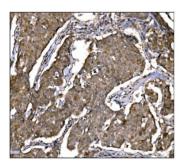


Figure 2. IHC analysis of Caspase-9/CASP9 using anti-Caspase-9/CASP9 antibody (A00080-6).
Caspase-9/CASP9 was detected in a paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-Caspase-9/CASP9 Antibody (A00080-6) 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 Strepavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

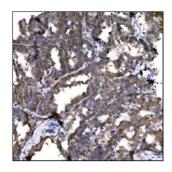
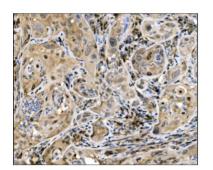


Figure 3. IHC analysis of Caspase-9/CASP9 using anti-Caspase-9/CASP9 antibody (A00080-6). Caspase-9/CASP9 was detected in a paraffin-embedded section of human ovarian cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-Caspase-9/CASP9 Antibody (A00080-6) 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 Strepavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

Figure 4. IHC analysis of Caspase-9/CASP9 using anti-Caspase-9/CASP9 antibody (A00080-6).





Caspase-9/CASP9 was detected in a paraffin-embedded section of human gallbladder adenocarcinoma 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-Caspase-9/CASP9 Antibody (A00080-6) overnight at 4°C. Biotinylated goat antirabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

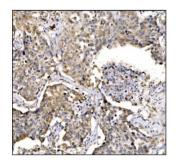


Figure 5. IHC analysis of Caspase-9/CASP9 using anti-Caspase-9/CASP9 antibody (A00080-6). Caspase-9/CASP9 was detected in a paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-Caspase-9/CASP9 Antibody (A00080-6) 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 Strepavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

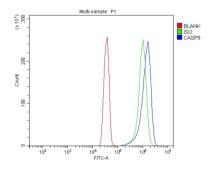


Figure 6. Flow Cytometry analysis of Hela cells using anti-Caspase-9/CASP9 antibody (A00080-6).

Overlay histogram showing Hela cells stained with A00080-6 (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 (A00080-6, 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 without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

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