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# Anti-Caspase-3 Antibody Picoband™ (monoclonal, 15G8)

Catalog # ABO14845

## Product Information

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| **Application** | WB, IHC, IF, ICC, FC |
| **Primary Accession** | P42574 |
| **Host** | Mouse |
| **Isotype** | Mouse IgG1 |
| **Reactivity** | Human |
| **Clonality** | Monoclonal |
| **Format** | Lyophilized |
| **Description** | Anti-Caspase-3 Antibody Picoband™ (monoclonal, 15G8) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human. |
| **Reconstitution** | Add 0.2ml of distilled water will yield a concentration of 500 □g/ml. |

## Additional Information

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| **Gene ID** | 836 |
| **Other Names** | Caspase-3, CASP-3, 3.4.22.56, Apopain, Cysteine protease CPP32, CPP-32, Protein Yama, SREBP cleavage activity 1, SCA-1, Caspase-3 subunit p17, Caspase-3 subunit p12, CASP3, CPP32 {ECO:0000303\|PubMed:7983002} |
| **Calculated MW** | 31608 |
| **Application Details** | Western blot, 0.1-0.5 □g/ml<br>Immunohistochemistry (Paraffin-embedded Section), 0.5-1 □g/ml<br>Immunocytochemistry/Immunofluorescence, 2 □g/ml, Human<br>Flow Cytometry, 1-3 □g/1x10^6 cells |
| **Subcellular Localization** | Cytoplasm. |
| **Tissue Specificity** | Highly expressed in lung, spleen, heart, liver and kidney. Moderate levels in brain and skeletal muscle, and low in testis. Also found in many cell lines, highest expression in cells of the immune system. |
| **Source** | Eukaryota |
| **Contents** | Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3. |
| **Clone Names** | Clone: 15G8 |
| **Immunogen** | E.coli-derived human Caspase-3 recombinant protein (Position: T67-D175). Human Caspase-3 shares 86% and 90% amino acid (aa) sequence identity with mouse and rat Caspase-3, respectively. |

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| **Cross Reactivity** | No cross-reactivity with other proteins. |
| **Storage** | 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. |

## Protein Information

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| **Name** | CASP3 |
| **Synonyms** | CPP32 {ECO:0000303\|PubMed:7983002} |
| **Function** | Thiol protease that acts as a major effector caspase involved in the execution phase of apoptosis (PubMed:18723680, PubMed:20566630, PubMed:23650375, PubMed:35338844, PubMed:35446120, PubMed:7596430). Following cleavage and activation by initiator caspases (CASP8, CASP9 and/or CASP10), mediates execution of apoptosis by catalyzing cleavage of many proteins (PubMed:18723680, PubMed:20566630, PubMed:23650375, PubMed:7596430). At the onset of apoptosis, it proteolytically cleaves poly(ADP-ribose) polymerase PARP1 at a '216-Asp-\|-Gly-217' bond (PubMed:10497198, PubMed:16374543, PubMed:7596430, PubMed:7774019). Cleaves and activates sterol regulatory element binding proteins (SREBPs) between the basic helix-loop-helix leucine zipper domain and the membrane attachment domain (By similarity). Cleaves and activates caspase-6, -7 and -9 (CASP6, CASP7 and CASP9, respectively) (PubMed:7596430). Cleaves and inactivates interleukin-18 (IL18) (PubMed:37993714, PubMed:9334240). Involved in the cleavage of huntingtin (PubMed:8696339). Triggers cell adhesion in sympathetic neurons through RET cleavage (PubMed:21357690). Also involved in axon pruning during brain development (By similarity). Cleaves DSG2 in response to apoptosis resulting in a loss of full length DSG2 at desmosome cell junctions and subsequent loss of cell-cell adhesion (PubMed:17559062). Also cleaves JUP in response to apoptosis (PubMed:17559062). Cleaves and inhibits serine/threonine-protein kinase AKT1 in response to oxidative stress (PubMed:23152800). Acts as an inhibitor of type I interferon production during virus-induced apoptosis by mediating cleavage of antiviral proteins CGAS, IRF3 and MAVS, thereby preventing cytokine overproduction (PubMed:30878284). Also involved in pyroptosis by mediating cleavage and activation of gasdermin-E (GSDME) (PubMed:35338844, PubMed:35446120). Cleaves XRCC4 and phospholipid scramblase proteins XKR4, XKR8 and XKR9, leading to promote phosphatidylserine exposure on apoptotic cell surface (PubMed:23845944, PubMed:33725486). Cleaves BIRC6 following inhibition of BIRC6-caspase binding by DIABLO/SMAC (PubMed:36758104, PubMed:36758106). |
| **Cellular Location** | Cytoplasm. |
| **Tissue Location** | Highly expressed in lung, spleen, heart, liver and kidney. Moderate levels in brain and skeletal muscle, and low in testis. Also found in many cell lines, highest expression in cells of the immune system. |

## Background

Caspase 3 is a caspase protein which interacts with Survivin, XIAP, CFLAR, Caspase 8, HCLS1, Deleted in Colorectal Cancer, TRAF3 and GroEL. This gene which is located on 4q35 encodes a protein that is 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 that undergo proteolytic processing at conserved aspartic residues to produce two subunits, large and small, that dimerize to form the active enzyme. It is the predominant caspase involved in the cleavage of amyloid-beta 4A precursor

protein, which is associated with neuronal death in Alzheimer's disease. And the caspase-3 activation in heart failure sequentially cleaves SRF and generates a truncated SRF that appears to function as a dominant-negative transcription factor. Additionally, the caspase-3 influence on bone mineral density should be considered in any in vivo application of caspase-3 inhibitors to the treatment of human disease. In erythroid precursors undergoing terminal differentiation, Hsp70 prevents active CASP3 from cleaving GATA1 and inducing apoptosis.

## Images

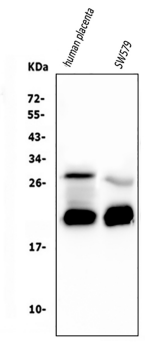



Figure 1. Western blot analysis of Caspase-3 using anti-Caspase-3 antibody (M00334-7).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human placenta tissue lysates;
Lane 2: human SW579 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 mouse anti-Caspase-3 antigen affinity purified monoclonal antibody (Catalog # M00334-7) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse 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 (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for Caspase-3 at approximately 20KD. The expected band size for Caspase-3 is at 32KD.

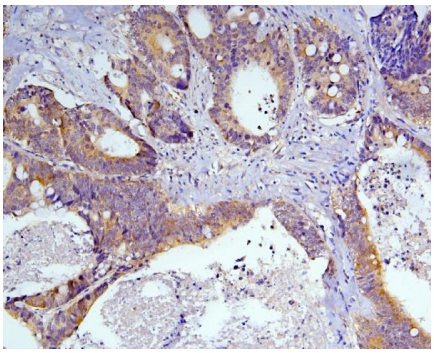


Figure 2. IHC analysis of Caspase-3 using anti-Caspase-3 antibody (M00334-7).
Caspase-3 was detected in paraffin-embedded section of human intestinal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Caspase-3 Antibody (M00334-7) overnight at 4°C. Biotinylated goat anti-mouse 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 # SA1021) with DAB as the chromogen.

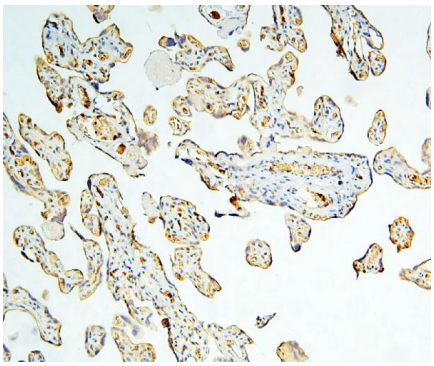


Figure 3. IHC analysis of Caspase-3 using anti-Caspase-3 antibody (M00334-7).
Caspase-3 was detected in paraffin-embedded section of human placenta tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Caspase-3 Antibody (M00334-7) overnight at 4°C. Biotinylated goat anti-mouse 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 # SA1021)

with DAB as the chromogen.

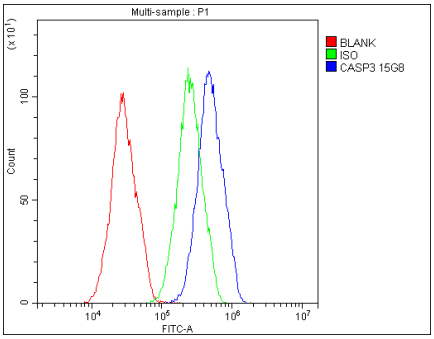



Figure 4. Flow Cytometry analysis of HepG2 cells using anti-Caspase-3 antibody (M00334-7).
Overlay histogram showing HepG2 cells stained with M00334-7 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Caspase-3 Antibody (M00334-7, 1 µg/1x$10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x$10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x$10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

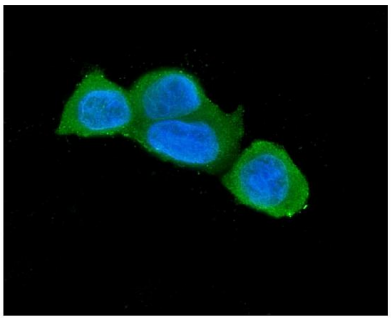


Figure 5. IF analysis of Caspase-3 using anti-Caspase-3 antibody (M00334-7).
Caspase-3 was detected in immunocytochemical section of MCF7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-Caspase-3 Antibody (M00334-7) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) 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.

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