

## Product datasheet for **AM26625AF-N**

### Caspase 3 (CASP3) Mouse Monoclonal Antibody [Clone ID: 1F9]

#### Product data:

Product Type:	Primary Antibodies
Clone Name:	1F9
Applications:	IHC, WB
Recommended Dilution:	<b>Western blot:</b> 1 µg/ml for chemiluminescence detection system. <b>Immunohistochemistry on Paraffin Sections:</b> 10 µg/ml. For details see protocols below. Not recommended for Immunoprecipitation.
Reactivity:	Human
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Human recombinant caspase 3
Specificity:	This antibody reacts with Human Caspase 3 (32kDa). Other species not tested.
Formulation:	PBS containing 50% glycerol, pH 7.2 State: Azide Free State: Liquid Ig fraction Preservative: None
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Upon receipt, store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	caspase 3
Database Link:	<a href="#">Entrez Gene 836 Human P42574</a>



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<b>Background:</b>	Caspase-3 (also known as CPP32, Yama, apopain) is a key member of the caspase family of cysteine proteases. Caspase-3 exists in cells as an inactive 32 kDa proenzyme. During apoptosis procaspase-3 is processed at aspartate residues by self-proteolysis and/or cleavage by upstream caspases, such as caspase-6, -8, or -9. The processed form of caspase-3 consists of large (17 kDa) and small (12 kDa) subunits which associate to form the active tetrameric enzyme tetramer (a pair of heterodimers). The active caspase-3 proteolytically cleaves and activates other caspases, as well as relevant targets in the cells (e.g., PARP, SREBPs, and DFF). Activation of procaspase-3 stands at a point of convergence for the two major types of apoptosis signaling pathways—those linked to cell surface death receptors and those linked to mitochondrial release of cytochrome c.
<b>Synonyms:</b>	CASP-3, CASP3, CPP32, CPP-32, Yama protein, Apopain, SCA-1, SCA1
<b>Note:</b>	This product was originally produced by MBL International.

Protocol: **SDS-PAGE & Western Blotting**

- 1) Wash the cells 3 times with PBS and suspend with 10 volumes of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
- 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make an 8 mg/mL solution.
- 3) Mix the sample with an equal volume of Laemmli's sample buffer.
- 4) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
- 5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for specific transfer procedure.
- 6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
- 7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the APPLICATIONS for 1 hour at room temperature. (The optimal antibody concentration will depend on the experimental conditions.)
- 8) Wash the membrane with PBS (5 minutes x 6 times).
- 9) Incubate the membrane with the 1:10,000 POD-conjugated anti-mouse IgG diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 10) Wash the membrane with PBS (5 minutes x 6 times).
- 11) Wipe excess buffer from the membrane, then incubate it with appropriate chemiluminescence reagents for 1 minute. Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
- 12) Expose to X-ray film in a dark room for 5 minutes. Develop the film as usual. The conditions for exposure and development may vary.

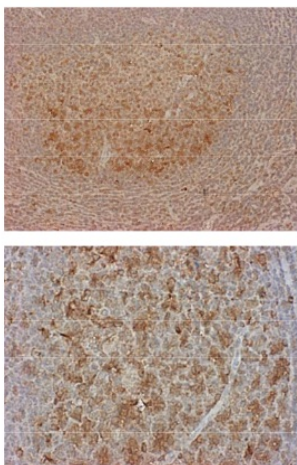
Positive controls for Western blotting; Raji, HL60, A431, HPB-ALL, KG-1

**Immunohistochemical staining for paraffin-embedded sections: SAB method**

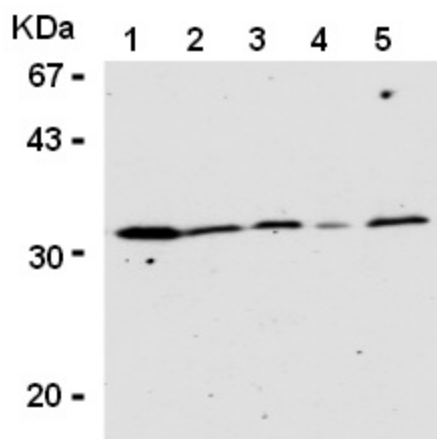
- 1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
- 2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
- 3) Wash the slides with PBS 3 times for 3-5 minutes each.
- 4) Heat treatment Heat treatment by microwave oven: Place the slides put on staining basket in 500 mL beaker with 500 mL citrate buffer (pH 6.5). Cover the beaker with plastic wrap, then process the slides 2 times for 10 minutes each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.
- 5) Remove the slides from the citrate buffer and cover each section with 3% H<sub>2</sub>O<sub>2</sub> for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
- 6) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent for 5 minutes to block non-specific antibody staining. Do not wash.
- 7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggested in the APPLICATIONS.
- 8) Incubate the sections for 1 hour at room temperature.
- 9) Wash the slides 3 times in PBS for 5 minutes each.
- 10) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody. Incubate for 10 minutes at room temperature. Wash as in step 9).
- 11) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase. Incubate for 10 minutes at room temperature. Wash as in step 9).
- 12) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40  $\mu$  L of 30% H<sub>2</sub>O<sub>2</sub> in 150 mL PBS.
- \* DAB is a suspected carcinogen and must be handled with care. Always wear gloves.
- 13) Wash the slides in water for 5 minutes.
- 14) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
- 15) Now ready for mounting.

Positive control for Immunohistochemistry; Tonsil

## Product images:



Immunohistochemical detection of caspase-3 on human tonsil paraffin embedded section with AM26625AF-N.



Western blot analysis of Caspase-3 expression in Raji (1), HL60 (2), A431 (3), HPB-ALL (4) and KG-1 (5) using AM26625AF-N.