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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:	**Western blot:** 1 µg/ml for chemiluminescence detection system. **Immunohistochemistry on Paraffin Sections:** 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:	Entrez Gene 836 Human P42574

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Background: 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 proteolyti cally 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.

Synonyms: CASP-3, CASP3, CPP32, CPP-32, Yama protein, Apopain, SCA-1, SCA1

Note: 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 μ L 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 2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture'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.

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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 beak er at room temperature for about 40 minutes.
5) Remove the slides from the citrate buffer and cover each section with 3% H_2O_2 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 temper ature. Wash as in step 9).
12) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 μ L of 30% H_2O_2 in 150 mL PBS.
* DAB is a suspected carcinoge n 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

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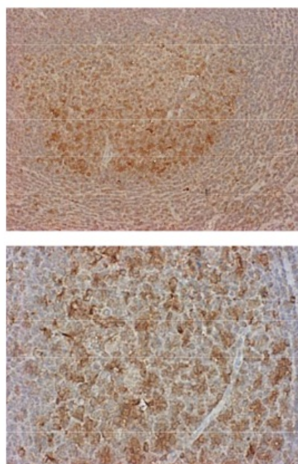
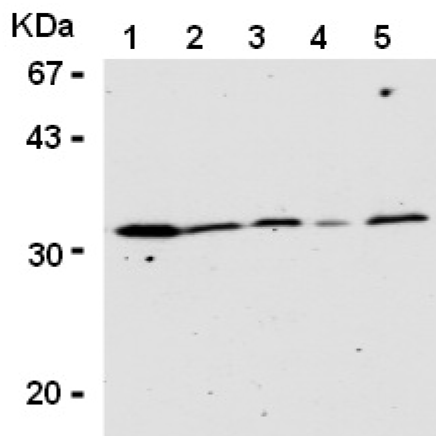
Product images:



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.

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