

Anti-Caspase 3 (Cleaved)

Rabbit polyclonal antibody
Reference: AP10070; AP10070C



1 of 2

INTENDED USE AND PRESENTATION:

For research use only.

AP10070, 7 ml. Prediluted antibodies in a synthetic organic linear polymer buffer solution (pH 7.4), with carrier protein and preservative for stabilisation "READY TO USE"

AP10070C, 1 ml. Concentrated antibodies with carrier protein and preservative for stabilisation.

SUMMARY, EXPLANATION AND LIMITATIONS:

A unique family of Cysteine proteases has been described that differs in sequence, structure and substrate specificity from any previously described protease family. This family, CED-3/caspase-1, is comprised of caspase-1, caspase-2, caspase-3, caspase-4, caspase-6, caspase-7 (also designated Mch3, ICE-LAP3 or CMH-1), caspase-9 and caspase-10. CED-3/caspase-1 family members function as key components of the apoptotic machinery and act to destroy specific target proteins which are critical to cellular longevity. Poly (ADP-ribose) polymerase plays an integral role in surveying for DNA mutations and double strand breaks. Caspase-3, caspase-7 and caspase-9, but not caspase-1, have been shown to cleave the nuclear protein PARP into an apoptotic fragment. Caspase-6, but not caspase-3, has been shown to cleave the nuclear lamins which are critical to maintaining the integrity of the nuclear envelope and cellular morphology. Caspase-10 has been shown to activate caspase-3 and caspase-7 in response to apoptotic stimuli.

Immunohistochemistry (IHC) is a complex technique in which immunological and histological detection methods are combined. In general, the manipulation and processing of tissues before immunostaining, especially different types of tissue fixation and embedding, as well as the nature of the tissues themselves may cause inconsistent results (Nadji and Morales, 1983). Endogenous pseudoperoxidase and peroxidase activity or endogenous biotin and alkaline phosphatase activity can cause non-specific staining results depending on the detection system used. Tissues that contain Hepatitis B surface antigen (HBsAg) can produce false positives when using HRP detection systems (Omata et al, 1980). Insufficient contrast staining and/or improper mounting of the sample may influence the interpretation of results.

Isotype: IgG

Immunogen: Synthetic peptide corresponding to residues adjacent to Asp175 in N-terminus of human caspase-3 (KLH).

Staining pattern: Cytoplasmic.

The interpretation of the stain results is the full responsibility of the user. Any experimental result must be confirmed by a medically established diagnostic product or procedure.

Positive control: Tissue sample from colon or tonsil.

External negative control: Tissue sample homologous to the test sample incubated with an antibody isotype not specific for caspase 3 (Cleaved).

APPLICATIONS:

This antibody is designed for the specific localization of human caspase 3 (cleaved) using IHC techniques in formalin-fixed, paraffin-embedded tissue sections.

PRODUCT COMPOSITION:

Rabbit immunoglobulin IgG, polyclonal, obtained from fraction of rabbit antiserum and purified by affinity. The preparation contains saline buffer, stabilising and carriers proteins, and sodium azide as a preservative.

METHODS AND PROCEDURE:

Principles of the procedure: The demonstrations of antigens by IHC is a sequential procedure with several steps involving first the application of a specific antibody for the antigen of interest (primary antibody), then a secondary antibody which joins to the first, an enzyme complex, and the addition of a chromogenic substrate. The sample is washed between each step. Enzymatic activation of the chromogenic substrate creates a visible product where the antigen is located. The results are interpreted using a light microscope. The primary antibody can be used both in manual IHC and with automated immunostainers.

Specimen: Paraffin-embedded tissue samples should be used. Western blot techniques are not recommended.

Staining procedure:

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|---|----------------------------|
| Antigen retrieval | HIER Citrate Buffer pH 6.5 |
| Working dilution (only for concentrates) | 1:50-1:150 |
| Incubation | 30 min; RT |
| Control Tissue | Colon or Tonsil |

Amplification and development of the immunostaining: follow standard procedure and the recommendations given by the manufacturer for the materials used. In the case of using automated immunostainers, use the specified buffers and materials for each instrument.

See our web site at www.gennova-europe.com for detailed protocols ancillary reagents and support products.

REQUIRED MATERIALS BUT NOT SUPPLIED:

All reagents, materials, and laboratory equipment for IHC procedures are not provided with this antibody. This includes adhesive slides and cover slips, positive and negative control tissues, Xylene or adequate substitute, ethanol, distilled H₂O, heat pretreatment equipment (pressure cooker, steamer, microwave), pipettes, Coplin jars, glass jars, moist chamber, histological baths, negative control reagents, counter-staining solution, mounting materials, and microscope.

Buffered solutions for antigen retrieval, enzyme treatments, highly sensitive detection systems, and other auxiliary reagents are available from Genova Scientific.

STORAGE AND STABILITY:

Store at -20 °C until the expiration date printed on product label. Do not use after the expiration date. If fresh solutions



Catalog number



Batch code



Research use only



Temperature limitation



Expiration date



Manufacturer



See instruction for use



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2 of 2

are required, these must be prepared immediately prior to use, and will be stable for at least one day at room temperature (20-25°C). Unused portion of antibody preparation should be discarded after one day. If the product is stored under different conditions from those stipulated in these technical indications, the new conditions must be verified by the user.

Gennova Scientific guarantees that the product will maintain all of the described characteristics from the production date until the expiration date, as long as the product is stored and used as recommended. No other guarantees are provided. Under no circumstances is Gennova Scientific obliged to cover damages caused by use of this reagent.

TROUBLESHOOTING:

If unusual staining is observed or any other deviations from the expected results, please read these instructions carefully, along with the instructions from the detection system. If this does not solve the problem, please contact Gennova Scientific's technical support department or your local distributor.

PRECAUTIONS:

Use only by qualified personnel.

Use proper protective equipment in order to avoid contact with reagents and samples in the eyes, skin, and mucosal tissues. In case of contact with sensitive areas, immediately flush the affected area with water. Avoid microbial contamination of the reagent, as this may produce nonspecific staining results. This antibody contains sodium azide (NaN_3), used as a stabilising agent, which is not considered to be a hazardous material in the concentration used. Concentration of sodium azide in drainage pipes made of lead or copper can cause the formation of highly explosive metallic azides. In order to avoid this, sodium azide must be disposed of along with a large volume of running water. Material safety data sheet (MSDS) for pure sodium azide is available upon request.

PERFORMANCE CHARACTERISTICS:

Gennova Scientific has performed studies to evaluate the functioning of these antibodies for use with standard detection systems, concluding that the product is both specific and sensitive for the antigen of interest.

BIBLIOGRAPHY:

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National Committee for Clinical Laboratory Standards (NCCLS). Protection of laboratory workers from infectious diseases transmitted by blood and tissue; proposed guideline. Villanova, PA 1991;7(9). Order code M29-P.
Nadji M, Morales AR. Immunoperoxidase, part 1: the techniques and its pitfall. *Lab Med* 1983; 14:767-770.
Omata M, Liew CT, Ashcavai M, Peters RI. Nonimmunologic binding of horseradish peroxidase to hepatitis B surface antigen. A possible source of error in immunohistochemistry. *Am J Clin Pathol.* May, 1980;73(5):626-632.

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|------------|------------------------|------------|-------------------------|------------|-------------------|
| REF | Catalog number | LOT | Batch code | RUO | Research use only |
| | Temperature limitation | | Expiration date | | |
| | Manufacturer | | See instruction for use | | |

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