



Ki-67 + Caspase-3

Prediluted Double Stain Antibody (4-Step)

Control Number: 901-240-112107

ISO
9001:2000
CERTIFIED

Catalog Number: PPM 240 DS AA, H

Description: 6.0, 25 ml, prediluted

Dilution: Ready-to-use

Diluent: N/A

Intended Use:

For In Vitro Diagnostic Use

Summary and Explanation:

The Ki-67 nuclear antigen is associated with cell proliferation. It is found throughout the cell cycle that includes the G1, S, G2, and M phases; but not the (GO) phase. This antibody cocktail was designed to provide information on cell death versus cell proliferation rates.

Apoptosis is a process, in which cells activate an intrinsic suicide mechanism that destroys themselves. The proteases that mediate this execution are called caspases (cysteiny-l-aspartic acid proteases). Cleaved caspase-3 detects endogenous levels of the large fragment (17/19 kDa) of activated caspase-3 resulting from cleavage adjacent to (Asp175). Activation of caspase-3 requires proteolytic processing of its inactive zymogen into activated p17 and p12 subunits. Cleavage of caspase-3 requires aspartic acid at the P1 position. This antibody does not cross react with other cleaved caspases. Cleaved caspase-3 is an affinity purified rabbit polyclonal antibody. This antibody cocktail of Ki-67 and Caspase-3 has been optimized with BIOCARE's Decloaking Chamber, Double Stain Kit # 2 and chromogens.

Principle of Procedure:

Antigen detection, in tissues and cells, is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a universal, affinity-purified, secondary antibody is added to bind to the primary antibody. An enzyme label is then added to bind to the secondary antibody; this detection of the bound antibody is evidenced by a colorimetric reaction.

Source: Mouse monoclonal and Rabbit Polyclonal

Species Reactivity: Human; others not tested

Clone: DVB-2

Isotype: IgG₁ (Ki-67 only)

Epitope/Antigen: Ki-67 and Caspase-3

Cellular Localization:

Ki-67: nuclear (brown) and Caspase-3: cytoplasmic (red)

Positive Control: Tonsil or colon cancer

Normal Tissue: Tonsil or colon

Abnormal Tissue: Breast and colon carcinomas

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative.

Storage and Stability:

Store at 2°C to 8°C. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Protocol Recommendations

Peroxide Block:

If using an HRP system, block for 5 minutes with BIOCARE's PEROXIDAZED 1.

Pretreatment Solution (recommended): Borg or Diva

Pretreatment Protocol:

Heat Retrieval Method:

Retrieve sections under pressure using BIOCARE's Decloaking Chamber, followed by a wash in distilled water. Alternatively, steam tissue sections for 45-60 minutes. Allow solution to cool for 20 minutes then wash in distilled water.

Protein Block:

Incubate for 10-15 minutes at RT with BIOCARE's Background Sniper.

Primary Antibody: Incubate for 60 minutes at RT.

Double Stain Detection:

Incubate for 30 minutes at RT using BIOCARE's Double Stain Kit #2.

Chromogen (1): Incubate for 5 minutes at RT when using BIOCARE's Betazoid DAB.

Chromogen (2):

Incubate for 10 minutes at RT with BIOCARE's Vulcan Fast Red.

Counterstain:

Rinse with deionized water. Incubate for 30-60 seconds with Tacha's Automated Hematoxylin. Rinse with deionized water. Apply Tacha's Bluing solution for 1 minute.

Technical Note:

This antibody has been standardized with BIOCARE's Double Stain Kit #2. It can also be used on an automated staining system. Use TBS buffer for washing steps.

Performance Characteristics:

The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to: fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of BIOCARE products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

Quality Control:

Refer to NCCLS Quality Assurance for Immunocytochemistry approved guidelines, December 1999 MM4-A Vol.19 No.26 for more information about Tissue Controls.

Precautions:

This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC.

Sodium azide (NaN₃) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for disease control, 1976, National Institute of Occupational Safety and Health, 1976)

Specimens, before and after fixation and all materials exposed to them, should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water.

Microbial contamination of reagents may result in an increase in nonspecific staining. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change. The MSDS is available upon request.

Troubleshooting:

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact BIOCARE's Technical Support at 1-800-542-2002.





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Limitations and Warranty:

There are no warranties, expressed or implied, which extend beyond this description. BIOCARE is not liable for property damage, personal injury, or economic loss caused by this product.

References:

1. Gown AM, Willingham MC. Improved detection of apoptotic cells in archival paraffin sections: Immunohistochemistry using antibodies to cleaved caspase 3. *J Histochem Cytochem* 2002 Apr;50(4):449-54
2. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
3. 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.

