Caspase-3 (Cleaved)

Concentrated and Prediluted Polyclonal Antibody
901-229-080217

BIOCARE MEDICAL

Catalog Number:	**CP 229 A, B, C**	**PP 229 AA**
Description:	0.1, 0.5, 1.0 ml, concentrated	6.0 ml, prediluted
Dilution:	1:100-1:200	Ready-to-use
Diluent:	Da Vinci Green	N/A

Intended Use:
For In Vitro Diagnostic Use
Caspase-3 (Cleaved) is a rabbit polyclonal antibody that is intended for laboratory use in the qualitative identification of caspase-3 protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:
Apoptosis has gained central importance in the study of many biological processes, including neoplasia, neurodegenerative diseases, and development. Caspases (cysteinyl-aspartic acid proteases) play a key role in the execution of apoptosis. Activation of caspase-3 requires proteolytic processing of its inactive zymogen into active p17 and p12 subunits. Caspase-3 (Cleaved) detects the large fragment (17/19 kDa) of activated caspase-3 resulting from cleavage adjacent to (Asp175). This antibody does not cross-react with other cleaved caspases.

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.

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, an enzyme labeled polymer is added to bind to the primary antibody. The detection of the bound antibody is evidenced by a colorimetric reaction.

Source: Rabbit polyclonal
Species Reactivity: Human and mouse
Clone: N/A
Isotype: N/A
Total Protein Concentration: ~10 mg/ml. Call for lot specific Ig concentration.
Epitope/Antigen: Caspase-3
Cellular Localization: Cytoplasmic / nuclear
Positive Control: Tonsil or colon cancer

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Supplied As: Buffer with protein carrier and preservative

Storage and Stability:
Store at 2ºC to 8ºC. Do not use after expiration date printed on vial. If reagents arestored 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 2ºC to 8ºC.

Protocol Recommendations:

Peroxide Block:
Block for 5 minutes with Biocare's Peroxidazed 1.

Pretreatment Solution (recommended): Reveal

Protocol Recommendations Cont'd:

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 10 minutes then wash in distilled water.

Protein Block (Optional): Incubate for 5-10 minutes at RT with Biocare's Background Punisher.
Primary Antibody: Incubate for 30 minutes at RT
Probe: N/A
Polymer: Incubate for 30 minutes at RT with a secondary-conjugated polymer.

Chromogen:
Incubate for 5 minutes at RT with Biocare's DAB - OR - Incubate for 5-7 minutes at RT with Biocare's Warp Red.

Counterstain:
Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

Technical Note:
This antibody has been standardized with Biocare's MACH 4 detection system. Use TBS buffer for washing steps.

Limitations:
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. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

Quality Control:
Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2). CLSI Wayne, PA, USA (www.clsi.org). 2011

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References:
1. Gown AM, Willingham MC. Improved detection of apoptic cells in archival paraffin sections: Immunohistochemistry using antibodies to cleaved capsase 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. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE

Rev: 062117

EC REP EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080