

## Rabbit anti-Caspase-3 (cleaved)

Cat. No.: RBK009-05 (0.5 ml Concentrate)

### Instructions for use

#### Intended use

This antibody is designed for the specific localisation of Caspase-3 (Caspase-3 cleaved) in formalin-fixed, paraffin-embedded tissue sections. Anti-Caspase-3 (cleaved) antibody is intended for research use only.

#### Specifications

<b>Specificity:</b>	Caspase-3 (cleaved)
<b>Clone:</b>	polyclonal
<b>Isotype:</b>	Rabbit Ig
<b>Species reactivity:</b>	Human +, mouse +, rat +, others not tested

#### Summary and Description

Caspases (cysteine aspartate-specific proteases) are cysteine proteases. They are cleaving protein chains specifically behind an aspartate residue. At the moment, 15 different caspases are known.

In a cascade of caspase reactions, caspase-8 is the first and therefore called initiator caspase. Caspase-8 activates other caspases (i.e. caspase-3, -6, and -7) through proteolytic cleavage. The activation of this so-called effector caspases leads in the end to biochemical and morphological apoptosis.

Caspase-3, also called CPP-32, apoptain, Yama or SCA-1, has in its inactivated form as procaspase-3 a molecular weight of 32 kDa. In apoptotic cells this protein is cleaved into two fragments, p12 and p17.

This anti-Caspase-3 (cleaved) antibody detects an epitope of the larger fragment of the activated (cleaved) caspase-3. It reacts neither with procaspase-3 nor with another activated caspase. The presence of activated (cleaved) caspase-3 is considered to be specific for apoptotic cells. That's why detection of cleaved caspase-3 is an excellent marker for apoptosis in immunohistochemistry.

#### Reagent provided

Rabbit polyclonal antibody in phosphate buffer with carrier protein and preservative for stabilisation in the following formats:

**Concentrate:** 0.5 ml (Cat. No. RBK009-05)

#### Dilution of primary antibody

Dilution of Zytomed Systems' concentrated antibody depends on the detection system used. The final working dilution must always be determined by the user. The elaboration of staining protocol should be done by an experienced specialist. For Zytomed Systems' recommendations see chapter 'Staining procedure'.

#### Storage and handling

The antibody should be stored at 2-8°C without further dilution.

Dilutions of the concentrated antibody should be done in a suitable antibody dilution buffer (e.g. ZUC025 from Zytomed Systems). The diluted antibody should be stored at 2-8°C after use. The stability of this working solution depends on various parameters and has to be confirmed by appropriate controls.

The antibody provided is suitable for use until the expiry date indicated on the label, if stored at 2-8°C. Do not use product after the expiry date. Positive and negative controls should be run simultaneously with all specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Zytomed Systems' technical support or your local distributor.

#### Precautions

Use through qualified personnel only.

Wear protective clothing to avoid contact of reagents and specimens with eye, skin and mucous membranes. If reagents or specimens come in contact with sensitive area, wash with large amounts of water.

Microbial contamination of the reagent must be avoided, since otherwise non-specific staining may occur.

Sodium azide ( $\text{NaN}_3$ ), used for stabilisation, is not considered hazardous material in the concentration used. Reaction of sodium azide with lead or copper in drainage pipes can result in the formation of highly explosive metallic azides.

Sodium azide should be discarded in a large volume of running water to avoid formation of deposits. A material safety data sheet (MSDS) for the pure substance is available upon request.

## Staining procedure

Refer to the following table for conditions specifically recommended for this antibody. Also refer to detection system data sheets for guidance on specific staining protocols or other requirements.

Parameter	Zytomed Systems recommendations
* Pre-treatment	Heat Induced Epitope Retrieval in Citrate Buffer pH 6.0 (ZUC028)
* Control tissue	Tonsil or colon carcinoma
* Working dilution	1:50 – 1:200 (for concentrated antibodies only)
* Incubation time	60 minutes

## Quality control

The recommended positive control tissues for this antibody are tonsil or colon carcinoma. We recommend carrying out a positive and a negative control with every staining run. Please refer to the instructions of the detection system for guidance on general quality control procedures.

## Troubleshooting

If you observe unusual staining or other deviations from the expected results please read these instructions carefully, refer to the instructions of the detection system for relevant information or contact your local distributor.

## Expected results

This antibody stains positive in cells with activated (cleaved) Caspase-3 in formalin-fixed, paraffin-embedded tissue sections. Activated Caspase-3 is detectable in the cytoplasm; more often a perinuclear localisation occurs, which seems to be a nuclear staining when observed by light microscopy.

Interpretation of the staining results is solely the responsibility of the user. Any experimental result should be confirmed by a medically established diagnostic procedure.

## Limitations of the Procedure

Immunohistochemistry is a complex technique involving both histological and immunological detection methods. Tissue processing and handling prior to immunostaining, for example variations in fixation and embedding or the inherent nature of the tissue can cause inconsistent results (Nadji and Morales, 1983). Endogenous peroxidase, alkaline phosphatase or biotin may cause non-specific staining depending on the detection system used. Tissues containing Hepatitis B Surface Antigen (HBsAg) may give false positive results with HRP (horse radish peroxidase) detection systems (Omata *et al*, 1980). Inadequate counterstaining and mounting can influence the interpretation of the results. Zytomed Systems warrants that the product will meet all requirements described from its shipping date until the expiry date is reached, if the product is stored and utilised as recommended. No additional guarantees can be given. Under no circumstances shall Zytomed System be liable for any damages arising out of the use of the reagent provided.

## Performance characteristics

Zytomed Systems has conducted studies to evaluate the performance of the antibody for use with a standard detection system. The product has been found to be sensitive and specific to the antigen of interest with minimal or no cross-reactivity.

## Bibliography

Gown AM and Willingham MC. J Histochem Cytochem 50:449-454, 2002  
Holubec H et al. J Histochem Cytochem 53:229-235, 2005  
Duan WR et al. J Pathol 199:221-228, 2003  
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


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Explanation of the symbol on the product label:

 REF	Bestellnummer Catalog Number Reference du catalogue	 LOT	Chargenbezeichnung Batch Code Code du lot	 Hersteller / Manufacturer / Fabricant  Zytomed Systems GmbH Anhaltiner Straße 22 D-14163 Berlin Deutschland Tel: (+49) 30-804 984 990 Fax: (+49) 30-804 984 999 <a href="mailto:info@zytomed-systems.de">info@zytomed-systems.de</a> <a href="http://www.zytomed-systems.de">www.zytomed-systems.de</a>
	Verwendbar bis Use By Utiliser jusque	 IVD	In vitro Diagnostikum In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro	
	Gebrauchsanweisung beachten Consult Instructions for use Consulter les instructions d'utilisation		Lagerungstemperatur Temperature Limitation Limites de température	