For life science research only.
Not for use in diagnostic procedures.

M30 CytoDEATH

Mouse Monoclonal antibody (CloneM30) for the detection of apoptosis in epithelial cells (caspase cleavage product of cytokeratin 18), Formalin grade [1]

Cat. No. 12 140 322 001 50 tests
Cat. No. 12 140 349 001 250 tests

Version 09
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Store at +2 to +8°C

[1] "During apoptosis, caspases cleave intracellular proteins; the corresponding caspase cleaving sites are formalin resistant. Formalin grade antibodies recognize cleavage sites in apoptotic cells that are not accessible in normal cells, which allows for determination of caspase activity, even in formalin-fixed, paraffin embedded tissue."

1. What this Product Does

Content

Formulation: White lyophilizate.

The antibody has been lyophilized in the presence of proteinous stabilizers.

Antibody Type

Clone M30, mouse IgG_{2b}

Storage and Stability

The lyophilized antibody is stable at +2 to +8°C until the expiration date printed on the label.

The antibody stock solution is stable for 6 months at +2 to +8°C. Alternatively, it can be stored in aliquots at −15 to −25°C.

⚠ Repeated freezing and thawing should be avoided.

The antibody is shipped at +15 to +25°C.

Application

Use for determination of early apoptosis in cells and tissue sections. A specific epitope of cytokeratin 18, which is presented after cleavage by caspase, is detected.

- Immunocytochemistry
- Flow Cytometry
- Immunohistochemistry:
- paraffin embedded tissue sections (secondary enhancer reagents needed)
- cryostat sections

2. How to Use this Product

2.1 Procedure for Immunofluorescence in Cells and Flow Cytometry

The following procedure describes the detection of apoptosis with the M30 CytoDEATH antibody in immunofluorescence and flow cytometry.

If using other detection methods or sample material, the conditions may vary and have to be adapted.

Additional Reagents Required

- Anti-Mouse IgG Fluorescein (CHEMICON (Millipore) AP308F)
- PBS* (Cat. No. 11 666 789 001)
- Methanol
- Tween 20* (Cat. No. 11 332 465 001)

Preparation of Working Solutions

The following table lists additional working solutions required for the immunofluorescence and flow cytometry procedure.

Working solution	Composition	Storage/ stability	Use
Washing buffer	PBS containing 0.1 % Tween 20	4 weeks at +2 to +8°C	Washing step
Incubation buffer	PBS containing 1% BSA and 0.1% Tween 20	4 weeks at +2 to +8°C	Preparation of antibody working solution

Preparation of Antibody Working Solution

Reconstitute lyophilizate in 550 µl double dist. water to obtain an antibody stock solution.

For application, dilute the antibody stock solution in Incubation buffer (1:10 for Cat. No. 12 140 322 001, or 1:50 for Cat. No. 12 140 349 001).

⚠ The antibody solutions should be free of precipitate. If necessary, centrifuge the solutions at high speed prior to use.

Protocol

Please refer to the following table

❶	Wash cells with PBS.
❷	Fix cells in ice-cold pure methanol at −15 to −25°C for 30 min.
❸	Wash cells with Washing buffer twice.
❹	Remove Washing buffer. Incubate with 100 µl M30 CytoDEATH antibody working solution for 60 min at +15 to +25°C.
❺	Wash cells with Washing buffer twice.
❻	Incubate with Anti-mouse IgG-Fluorescein (10 µg/ml) for 30 min at +15 to +25°C.
❼	Wash cells with Washing buffer twice.
❽	**If you use...** → **THEN...** a fluorescence microscope → examine the cells on a slide. flow cytometry → dilute cells in 0.5 ml PBS and store the samples in the dark until analysis.

* *available from Roche Diagnostics*

2.2 Procedure for Immunohistochemistry

The following procedure describes the detection of apoptosis with the M30 CytoDEATH antibody in immunohistochemistry (Paraffin embedded tissue).

If using other detection methods or sample material, the conditions may vary and have to be adapted

Additional Reagents Required

For preparation of samples:

- Xylol
- Ethanol, 96%
- Ethanol, 70%
- Methanol/ H_2O_2 (3%)

For the immunohistochemistry procedure:

- Anti-mouse-Ig Biotin (CHEMICON AP302B)
- Streptavidin-POD* (Cat. No. 11 089 153 001)
- DAB substrate* (Cat. No. 11 718 096 001) or
- AEC
- PBS* (Cat. No. 11 666 789 001)
- Tween 20* (Cat. No. 11 332 465 001)
- Harries hematoxilin
- Kaisers glycerin gelatine
- Citric acid
- NaOH, 1 N

Preparation of Working Solutions

The following table lists the working solutions needed to perform the immunohistochemistry staining procedure.

Working Solution	Composition	Storage/ Stability	Use
Washing buffer	PBS containing 0.1% Tween 20	4 weeks at +2 to +8°C	Washing step
Incubation buffer	PBS containing 1% BSA and 0.1% Tween 20	4 weeks at +2 to +8°C	Preparation of antibody working solution and blocking step
Citric acid buffer	2g/l citric acid, pH 6 adjusted with 1 N NaOH	4 weeks at +2 to +8°C	Antigen retrieval

Preparation of Antibody Working Solution

Reconstitute lyophilizate in 550 µl to obtain an antibody stock solution. Dilute the antibody stock solution 1 : 10 for Cat. No. 12 140 322 001, or 1 : 50 for 12 140 349 001 in Incubation buffer.

⚠ The antibody solutions should be free of precipitate. If necessary, centrifuge the solution at high speed prior to use.

Preparation of Sample Material

Before you start with the immunohistochemical protocol dewax paraffin embedded tissue sections as described in the following table.

⚠ The section should not be allowed to dry during this procedure.

❶	Place paraffin embedded sections into an incubator at +37°C over night.
❷	Dewax formalin-fixed, paraffin-embedded tissue sections: ▪ 2 coplin jars of xylol (2–5 min), ▪ 2 coplin jars of ethanol (96%, dip 30×), ▪ 1 coplin jar of ethanol (70%, dip 30×), ▪ 1 coplin jar of methanol/ H_2O_2 (3%) for 10 min at +15 to +25°C.
❸	Rinse 10 min in double dist. water.

Protocol

Strictly follow the below mentioned method for antigen retrieval!

❶	▪ Prewarm Citric acid buffer by incubation in a microwave oven at 750 W until solution boils. ▪ Place tissue section slides in a slide rack and put them into the heated Citric acid solution. Turn microwave oven on (at 750 W) until solution boils. ▪ When the solution is boiling, turn the setting of the microwave oven to “keep warm” (about 100 W). ▪ Incubate at this setting for 15 min. ⚠ For optimal morphology we recommend to keep the solution shortly below the boiling point to avoid gas formation under the sections.
❷	Cool down the solution for 5 min at +15 to +25°C.
❸	Rinse 3× in PBS and incubate 2 min in a separate jar of PBS.
❹	Block section with Incubation buffer for 10 min at +15 to +25°C.
❺	Remove Incubation buffer and add 100 µl antibody working solution for 1 h at +15 to +25°C in a humid chamber.
❻	Wash slides in Washing buffer (use 3 separate jars and dip 30× into each jar).
❼	▪ Cover the preparation with 50–100 µl of Anti-mouse-Ig Biotin (1–2 µg/ml). ▪ Incubate for 30 min at +37°C in a humid chamber.
❽	Wash slides in Washing buffer (use 3 separate jars and dip 30× into each jar).
❾	▪ Cover the section with 100 µl of Streptavidin-POD (0.5 U/ml). ▪ Incubate for 30 min at +15 to +25°C in a humid chamber.
❿	Wash slides in Washing buffer (use 3 separate jars and dip 30× into each jar).
⓫	Incubate slides in a freshly prepared substrate solution (DAB or AEC) at +15 to +25°C until a clearly visible color develops (1–5 min). A negative control should not show any development of color during the incubation period.
⓬	Stop the reaction by extensive rinsing in double dist. water.
⓭	Subsequently, counterstain the preparation with Harries hematoxilin (dip shortly) and mount the section (with *e.g.* Kaisers glycerin gelatine).

3. Results

Immunohistochemistry

- Apoptotic cells are stained extensively inside the cytoplasm. Granular structures could be observed in the late phase of apoptosis.
- Staining of somehow preserved apoptotic fragments of CK18 could be observed inside blood vessels.
- Macrophages might exhibit positive staining due to phagocytosis of apoptotic cells or apoptotic bodies.
- If nuclear staining is observed, this is not correlated to apoptosis and is occasionally found in highly proliferating tissue (never observed in cell cultures). For reduction of this unspecific staining, use higher dilutions of the antibody.

Flow Cytometry

Following figure shows typical histograms of normal and apoptotic HeLa cells analyzed after M30 CytoDEATH, staining:

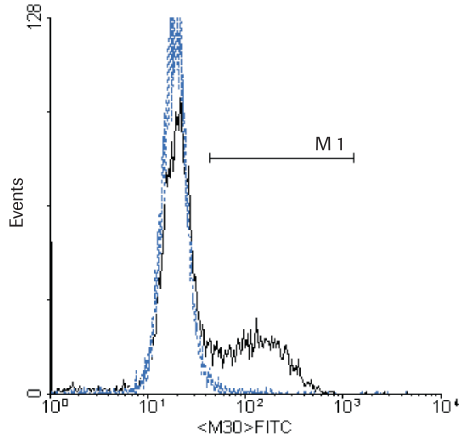

Fig. 1: HeLa cells were induced to apoptosis by treatment with 10 ng/ml TNF-α and 5 μg/ml Actinomycin D for 3 h at +37°C. After treatment, cells were stained with M30 CytoDEATH, according to the staining procedure under 3.2.2.
Dotted line = histogram of normal cells,
solid line = histogram of apoptotic cells.
Cells analyzed under marker M1 are apoptotic.

4. Troubleshooting

	Possible cause	Recommendation
Unspecific staining (*e.g.* nuclear staining)	Antibody concentration too high	Decrease concentration
Labeling index too high	Antibody concentration too high	Decrease concentration
Background too high	Washing steps too short	Use PBS/0.1% Tween 20 twice for 5 min each
Weak or no signal	Inadequate incubation time and temperature	Ensure that incubation intervals are correct, and that all reagents achieve the adequate temperature before testing
	Too low or no expression of Cytokeratin 18	As a control for the expression of Cytokeratin 18 in a particular sample it is possible to use the Anti-Cytokeratin No. 18 provided by CHEMICON (MAB3404).

5. Additional Information on this Product

Background Information

The Function of Caspases during Apoptosis

Apoptosis is a process, in which cells activate an intrinsic suicide mechanism that systematically destroys themselves. It proceeds through cleavage of native intracellular proteins. The proteases which mediate this execution are called caspases (**C**ysteinyl-**asp**artic acid prote**ases**). Caspases are expressed as zymogenes, which are activated by different apoptosis inducers. Once activated, a single caspase starts the activation of a cascade of caspases.

Technique for Detection of Apoptosis

Cytokeratins, in particular cytokeratin 18, are affected in early events of apoptosis (1). Furthermore, the antibody M30 CytoDEATH recognizes a specific caspase cleavage site within cytokeratin 18 that is not detectable in native CK18 of normal cells (2). Consequently, the M30 CytoDEATH is a unique tool for easy and reliable determination of very early apoptosis in single cells and tissue sections (3–7).

Product Characteristics

Preparation

To obtain monoclonal antibodies (moAb), Balb-c-mice received injections of a CK18 related peptide.

Eight weeks after the initial intraperitoneal injection, spleen cells of these Balb-c-mice were fused with P3-X63-Ag8.653 myeloma cells. Positive hybridomas were cloned by limiting dilution. The hybridoma supernatants were screened for reactivity with a biotin-labeled peptide. By immunostaining it was observed that one of the resulting hybrid cell lines (No. 30) produced antibodies, which reacted with cultured cells, that were neither vital nor necrotic.

Detailed studies showed that the moAb reacts specifically with apoptotic epithelial cells.

Purification of Antibody

The antibody was purified by ammonium sulfate precipitation followed by ion exchange chromatography, and diluted in 20 mM potassium phosphate, 50 mM NaCl, 1% BSA (w/v), and 0.01% methylisothiazolone (MIT) (v/v), pH 7.5.

Specificity

- The antibody binds to a caspase cleaved formalin-resistant epitope of the human cytokeratine 18 (CK18) cytoskeletal protein.
- The immunoreactivity of the M30 antibodies is confined to the cytoplasma of apoptotic cells.
- Nonspecific crossreactivity with nuclear antigens of highly proliferating cells can occur.

References

1 Caulin, C. et al. (1997) Caspase Cleavage of Keratin 18 and Reorganization of Intermediate Filaments during Epithelial Cell Apoptosis. *J. Cell Biol*. **138**, 1379-1394.

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3 Kronberger, B. et al. (2000). Hepatocellular proliferation in patients with chronic hepatitis C and persistently normal or abnormal aminotransferase levels. *J Hepatology* 2000 **33**, 640-647.

4 Yu, D-C. et al. (2001). Antitumor Synergy of CV787, a Prostate Cancer-specific Adenovirus, and Paclitaxel and Docetaxel. *Cancer Research* **61**, 517-525.

5 Michael-Robinson, J.M. et al. (2001) Tumour infiltrating lymphocytes and apoptosis are independent features in colorectal cancer stratified according to microsatellite instability status. *Gut 2001* **48**, 360-366.

6 Bantel, H. et al. (2001). Detection of elevated caspase activation and early apoptosis in liver diseases. *European Journal of Cell Biology* **80**, 230-239.

7 Roy, H. K. et al. (2001). Chemoprevention of intestinal tumorigenesis by nabumetone: induction of apoptosis and Bcl-2 downregulation. *British Journal of Cancer* 84 (**10)**, 1412-1416.

Quality Control

The antibody is function tested using a cellular model (HeLa cells treated with TNF-α/Actinomycin D).

6. Supplementary Information

6.1 Conventions

Text Conventions

To make information consistent and memorable, the following text conventions are used in this document:

Text Convention	Usage
Numbered instructions labeled ❶ ❷	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Diagnostics.

Symbols

In this document, the following symbols are used to highlight important information:

N	Important Note: Information critical to the success of the procedure or use of the product.

6.2 Ordering Information

Apoptosis-specific physiological change	Detection Mode/ Product	Pack Size	Cat. No.
DNA fragmentation	Gel Electrophoresis		
	Apoptotic DNA-Ladder Kit	20 tests	11 835 246 001
	In situ assay		
	In Situ Cell Death Detection Kit, TMR red	1 kit (50 tests)	12 156 792 910
	In Situ Cell Death Detection Kit, Fluorescein	1 kit (50 tests)	11 684 795 910
	In Situ Cell Death Detection Kit, AP	1 kit (50 tests)	11 684 809 910
	In Situ Cell Death Detection Kit, POD	1 kit (50 tests)	11 684 817 910
	Single reagents for TUNEL and supporting reagents		
	TUNEL AP	70 tests (3.5 ml)	11 772 457 001
	TUNEL POD	70 tests (3.5 ml)	11 772 465 001
	TUNEL Enzyme	2 × 50 µl (20 tests)	11 767 305 001
	TUNEL Label	3 × 550 µl (30 tests)	11 767 291 910
	TUNEL Dilution Buffer	20 ml	11 966 006 001
	ELISA		
	Cell Death Detection ELISA	1 kit	11 544 675 001
	Cell Death Detection ELISA^{PLUS}	1 kit (96 tests)	11 774 425 001
	Cell Death Detection ELISA^{PLUS}, 10×	1 kit	11 920 685 001
	Cellular DNA Fragmentation ELISA	1 kit (500 tests)	11 585 045 001
Cell membrane alterations	Microscopy or FACS		
	Annexin-V-Alexa 568	250 tests	03 703 126 001
	Annexin-V-Biotin	250 tests	11 828 690 001
	Annexin-V-Fluorescein	1 mg	11 975 595 001
	Annexin-V-FLUOS	250 tests	11 828 681 001
	Annexin-V-FLUOS Staining Kit	50 tests 250 tests	11 858 777 001 11 988 549 001
Enzymatic activity	Western Blot		
	Anti-Poly (ADP-Ribose) Polymerase	100 µl	11 835 238 001
	FIENA		
	Caspase 3 Activity Assay	1 kit	12 012 952 001
	Homogenous Caspases Assay, fluorometric	100 tests 1000 tests	03 005 372 001 12 236 869 001
	In situ Assay		
	M30 CytoDEATH (formalin grade)	50 tests 250 tests	12 140 322 001 12 140 349 001
	M30 CytoDEATH, Fluorescein	250 tests	12 156 857 001
	Anti-PARP Caspase Cleavage Product-Fluorescein	50 tests 250 tests	03 371 131 001 03 371 123 001
Expression of apoptosis-related proteins	Apoptosis Induction		
	Anti-Fas (CD95/APO-1)	1000 tests	11 922 432 001

6.3 Trademarks

All third party product names and trademarks are the property of their respective owners.

6.4 Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

6.5 Changes to Previous Version

Editorial Changes.

6.6 Disclaimer of License

For patent license limitations for individual products please refer to:
List of biochemical reagent products

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