

## M30 CytoDEATH\*

Cat. No. 12 140 322 001 50 tests

Cat. No. 12 140 349 001 250 tests

## M30 CytoDEATH, Fluorescein

Cat. No. 12 156 857 001 250 tests

|                   |  |
|-------------------|--|
| <b>Type</b>       | Monoclonal antibody, clone M30, IgG2b, mouse   |
| <b>Useful for</b> | Detection of apoptosis in epithelial cells and tissues (formalin grade)  |
| <b>Samples</b>    | Adherent cells, tissue samples (routinely fixed and paraffin-embedded tissue sections, cryostat sections)  |
| <b>Method</b>     | Detect apoptosis by applying the M30-antibody to fixed samples, then using secondary detection systems. Suitable for immunohistochemistry, immunocytochemistry, and flow cytometry |
| <b>Time</b>       | 2 h for immunofluorescence on cells, 3.5 h for staining of tissues (excluding dewaxing)  |

**Background:** During Apoptosis, vital intracellular proteins are cleaved. The proteases that mediate this process are called caspases (Cysteiny $\text{l}$ -aspartic acid proteases). Caspases are expressed as zymogenes, which are activated by different apoptosis inducers. Once activated, a single caspase activates a cascade of caspases.

Until recently cytokeratins, in particular cytokeratin 18, were not known to be affected by early events of apoptosis. Recently, it has been shown that the M30 antibody recognizes a specific caspase cleavage site within cytokeratin 18 that is not detectable in native CK18 of normal cells. Consequently, the M30 CytoDEATH antibody is a unique tool for the easy and reliable determination of very early apoptotic events in single cells and tissue sections.

**Significance of reagent:** Use the M30 CytoDEATH antibody for the determination of early apoptotic events in cells and tissue sections by detection of a specific epitope of cytokeratin 18 that is presented after cleavage by caspases.

**Test principle of M30 CytoDEATH**  
for formalin-embedded tissue involves:

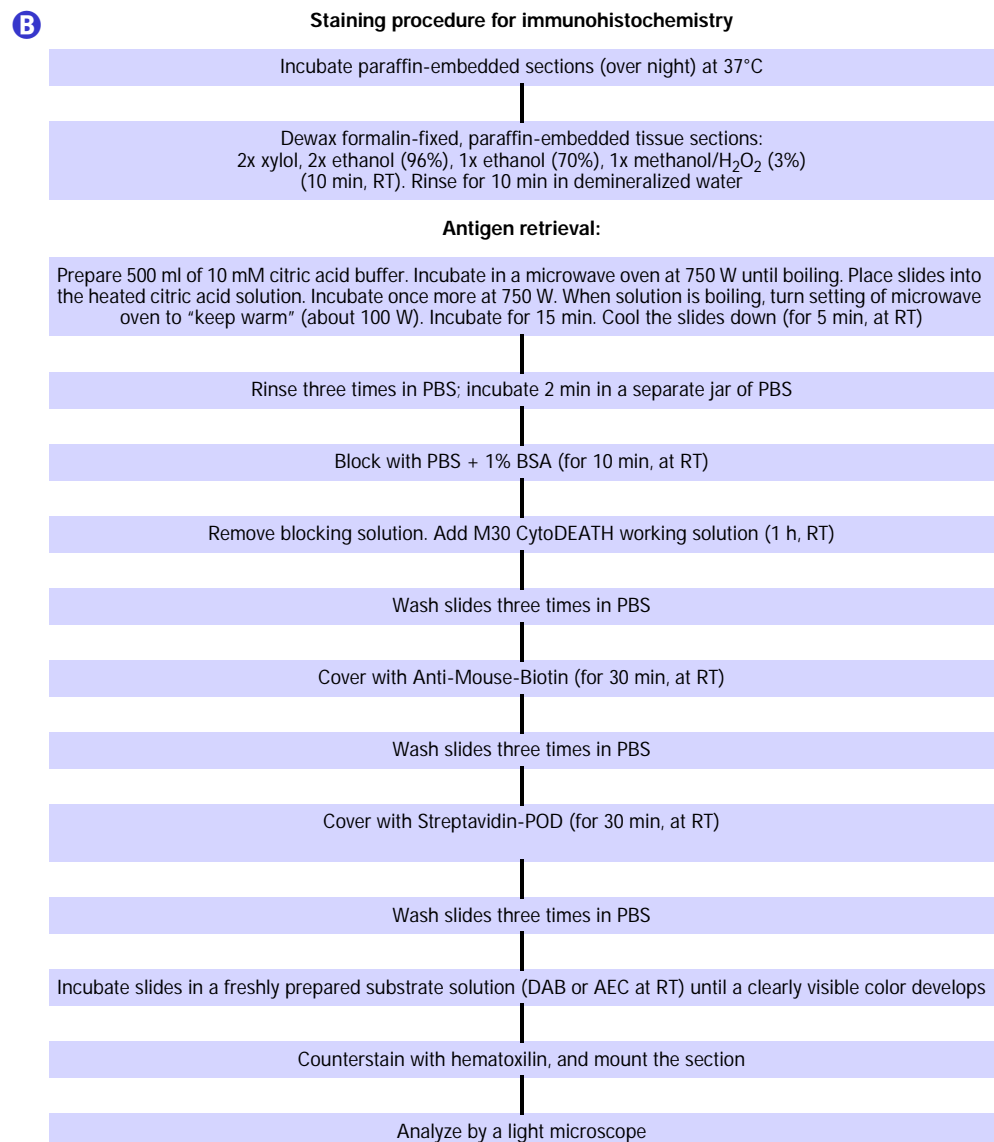
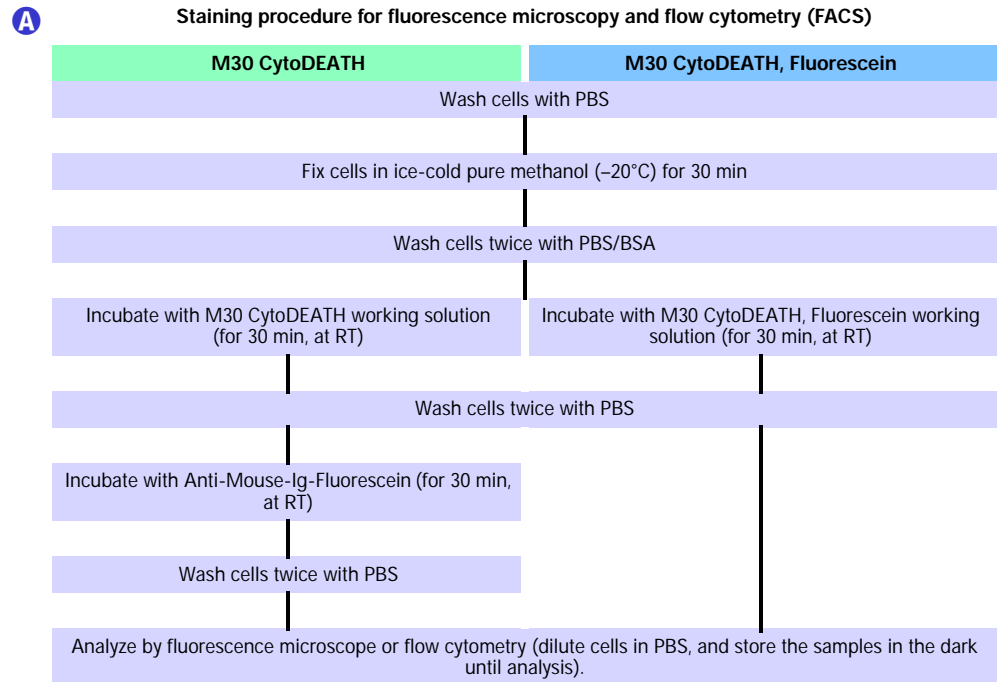
|  |  |
|--|--|
| 1 Dewax formalin-fixed, paraffin-embedded tissue sections. | 5 Add Streptavidin-POD.                |
| 2 Retrieve antigen by heating in citric acid buffer.       | 6 Add substrate solution (DAB or AEC). |
| 3 Add M30 antibody.  | 7 Counterstain with Harries hematoxin. |
| 4 Add Anti-Mouse-Biotin.                                   | 8 Analyze under a light microscope.    |

\* The M30 CytoDEATH antibody is made under a license agreement from BEKI AB/BEKI Diagnostics AB, Sweden.

for immunofluorescence and flow cytometry on cells  
for M30 CytoDEATH and M30 CytoDEATH, Fluorescein:

|  |   |
|--|---|
| 1 Fix cells.   | 3 Add Anti-Mouse-Ig-Fluorescein (not necessary for M30 CytoDEATH, Fluorescein). |
| 2 Add M30 CytoDEATH antibody or M30 CytoDEATH, Fluorescein | 4 Analyze under a fluorescence microscope or in FACS.                           |

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▲ Flow Chart 3: Assay procedure, M30 CytoDEATH, immunohistochemistry and cytometry.

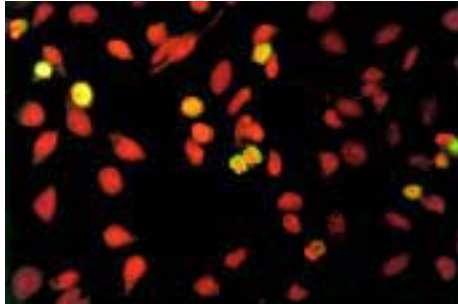
**Specificity:** The M30 CytoDEATH antibody binds to a caspase-cleaved, formalin-resistant epitope of the cytokeratin 18 (CK 18) cytoskeletal protein.

The immunoreactivity of the M30 CytoDEATH antibody confined to the cytoplasm of apoptotic cells.

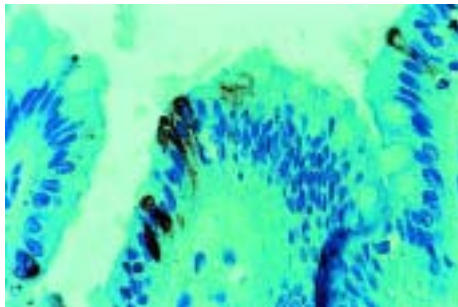
**Antibody supplied as:** Mouse monoclonal antibody (clone M30), lyophilized, stabilized. Formalin grade.

**Typical results:** see Figures 10–12.

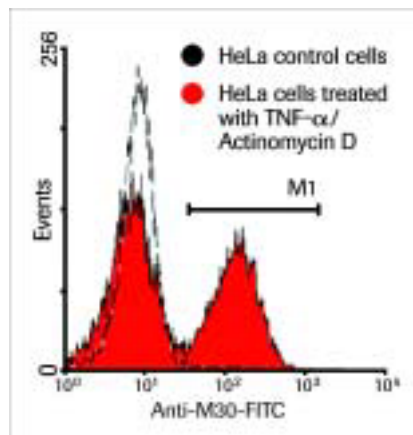
**Other applications:** For more examples of how the M30 CytoDEATH and M30 CytoDEATH, Fluorescein can be used in the lab, see Appendix, pages 131–132.



▲ Figure 10: Detection of apoptosis in HeLa cells, treated with TNF and Actinomycin D, using M30 CytoDEATH. Secondary detection with Anti-Mouse-Fluorescein and propidium iodide.



▲ Figure 11: Detection of apoptosis in human colon using M30 CytoDEATH (blue filter). Secondary detection with Anti-Mouse-Biotin, Streptavidin-POD and AEC as substrate, counterstained with hematoxylin.



▲ Figure 12: FACS analysis of apoptosis in HeLa cells, using M30 CytoDEATH, Fluorescein. White: untreated control cells. Red: Cells treated with TNF and Actinomycin D.