**M30 CytoDEATH™ Antibody**

- Easy-to-use: supplied in liquid format with simple standard staining protocol
- Highly reproducible results specific for epithelial apoptosis
- Allows sensitive detection of early and late stages of epithelial apoptosis
- Superior to TUNEL, ISEL, annexin V and anti-active caspase-labelling
- Sustained signal from cells at early to late stages of apoptosis

**CLONE:** M30  
**ISOTYPE:** Mouse IgG2b  
**REACTIVITY:** Human, mouse, rat, rabbit.

<table>
<thead>
<tr>
<th></th>
<th>ALX-804-590-T200</th>
<th>ALX-804-590B-T200</th>
<th>ALX-804-590F-T200</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>200 tests</td>
<td>200 tests</td>
<td>200 tests</td>
</tr>
<tr>
<td>APPLICATION:</td>
<td>FC, ICC, IHC (FS, PS), WB</td>
<td>2-Step IHC (FS, PS)</td>
<td>1-Step FC, 1-Step ICC</td>
</tr>
</tbody>
</table>

**M30 Apoptosense® ELISA Kit**

For specific, sensitive and quantitative measurement of epithelial apoptosis

- **in vitro:** High throughput drug screening, molecular pathway and target evaluation assays
- **in vivo:** Non-invasive assay using human blood samples for research in cancer, liver pathologies and sepsis

<table>
<thead>
<tr>
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<th>ALX-850-270-KI01</th>
<th>ALX-850-270-5001</th>
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<tbody>
<tr>
<td></td>
<td>1 Kit</td>
<td>5 Kits</td>
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</table>

**M65® ELISA Kit**

For rapid, sensitive and quantitative measurement of total epithelial cell death (apoptotic and necrotic) in human blood samples

<table>
<thead>
<tr>
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<th>ALX-850-310-KI01</th>
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<tbody>
<tr>
<td></td>
<td>1 Kit</td>
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</table>
M30 CytoDEATH™ Antibody

**ALX-804-590-T200**  
**M30 CytoDEATH™ – key advantages**

**BENEFITS**  
**FEATURES**

- Broad species reactivity  
The epithelial cytoskeleton protein cytokeratin 18 neo-epitope (CK18-NE) recognized by M30 CytoDEATH™ antibody is conserved between human, mouse, rat and rabbit. Other species not tested.

- Broad application range  
The M30 CytoDEATH™ antibody has been successfully used in Western blot, immunocytochemistry, flow cytometry and immunohistochemistry, including frozen and formalin-fixed, paraffin-embedded tissue sections.

- Recommended for formalin-fixed paraffin-embedded tissue  
Recommended for routinely fixed tissue samples. Retrograde studies are possible, even on archive material, as the M30 antigen is abundant and formalin-resistant.

- Convenient  
Easy to use standard protocol with M30 CytoDEATH™ antibody provided in a ready-to-use format.

**M30 CytoDEATH™ – BIOTIN**

**ALX-804-590B-T200**  
**Biotin – 200 tests**

**M30 CytoDEATH™ Biotin – additional advantages**

**BENEFITS**  
**FEATURES**

- Added convenience for immunohistochemistry  
Two-step tool for the detection of apoptosis in epithelial cells by immunohistochemistry. No additional anti-mouse IgG biotin conjugated secondary antibodies required. No background problems especially with samples from mouse or rat.

**M30 CytoDEATH™ – FLUORESCINE**

**ALX-804-590F-T200**  
**FITC – 200 tests**

**M30 CytoDEATH™ Fluorescein – additional advantages**

**BENEFITS**  
**FEATURES**

- Added convenience for flow cytometry and immunocytochemistry  
One-step tool for the detection of apoptosis in epithelial cells by flow cytometry and immunocytochemistry. No additional anti-mouse IgG fluorochrome-conjugated secondary antibodies required. No background problems especially with samples from mouse or rat.

Selection of CK18 positive human cell lines and tissues successfully analyzed with the M30 CytoDEATH™ antibody:

- Breast cancer: MDA-MB-231, MCF-7, HBL100
- Colon cancer: WiDr, HCT 116
- Cervical cancer: HeLa
- Embryonic kidney: Hek 293
- Head & neck cancer: SCC9, SCC25
- Prostate cancer: PC-3, LNCaP, DU 145

**M30 CytoDEATH™** is cited in more than 100 publications  
– visit our website for a comprehensive overview

**FIGURE:** Detection of apoptosis in a formalin-fixed and paraffin-embedded tissue section from a human colon cancer showing confined cytoplastic staining for CK18-NE using M30 CytoDEATH™. Secondary detection with anti-mouse IgG-biotin, streptavidin-POD and AEC as substrate, counterstained with hematoxylin.

**FIGURE:** Apoptosis of human cervical carcinoma HeLa cells treated with recombinant human TRAIL was quantified with M30 CytoDEATH™ in flow cytometry. Left) M30 CytoDEATH™-FITC; Right) M30 CytoDEATH™-GAM FITC. M30 CytoDEATH™ antibodies were used at 1μg/ml in a 100μl sample size.

**FIGURE:** Typical standard curve for M65™ ELISA Kit.

<table>
<thead>
<tr>
<th>M65® ELISA Kit</th>
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<tbody>
<tr>
<td>M65-Va lue (U/L)</td>
<td>0</td>
<td>50</td>
<td>125</td>
<td>250</td>
</tr>
<tr>
<td>Typical standard curve for M65™ ELISA Kit</td>
<td></td>
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</tr>
</tbody>
</table>

**EASY-TO-USE!**

**LITERATURE REFERENCES**

**Cancer Research**

**Liver Disease Research**

**Sepsis Research**

For updated prices and additional information visit www.alexis-biochemicals.com, contact your local distributor, or call +41 61 926 89 89.
**M 3 0  A p o p t o s e n s e ®  E L I S A K i t**

**M 3 0  A P O T O S E N S E ®  E L I S A K i t**

- **ALX-850-270-KI01** 1 Kit
- **ALX-850-270-5001** 5 Kits

Anti-Ck18 MAb precoated 96-well ELISA plate with M30-conjugated to HRP as detection antibody. For specific and sensitive quantification of caspase-cleaved, soluble CK18NE as an epithelial apoptosis (bio)marker in cell lysates, cell supernatants or human serum and plasma samples. Does not detect intact CK18.

For quantitative measurement of apoptosis in:
- Drug Screening & Discovery (in vitro)
- Identify Pro-apoptotic Drug Candidates
- Perform Dose-response Studies
- Perform Mechanistic Studies
- Assays with human blood samples (in vivo) in
  - Cancer Research
  - Septic Shock Research
  - Hepatic Injuries [Hepatitis, Cirrhosis] Research

**Note:** The CK18NE antigen is very stable in blood samples, which are stable for at least 12 months when stored at -80°C.

**M 6 5 ® E L I S A K i t**

**M 6 5 ® E L I S A K i t**

- **ALX-850-310-KI01** 1 Kit

Anti-Ck18 MAb precoated 96-well ELISA plate with M65-conjugated to HRP as detection antibody. For specific and sensitive quantification of intact CK18 and/or caspase-cleaved, soluble CK18-NE in human serum and plasma samples. Detects total epithelial cell death as a result of apoptosis plus necrosis.

In combination with M30 Apoptosense® ELISA Kit, M65® ELISA Kit is useful for the calculation of apoptotic versus necrotic cell death, i.e. to determine which mode of cell death led e.g. to carcinoma cell death.

**TECHNICAL NOTE**

**M 3 0  v e r s u s  T U N E L  v e r s u s a n t i - C a s p a s e - 3 ( a c t i v e )  A n t i b o d y**

**BENEFITS**

<table>
<thead>
<tr>
<th></th>
<th>M30</th>
<th>TUNEL</th>
<th>anti-CASP-3 AB</th>
</tr>
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<tbody>
<tr>
<td>Early and specific detection of apoptosis</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>Sustained signal from cells at early to late stages of apoptosis</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Specificity for epithelial (i.e. carcinoma) apoptosis</td>
<td>YES</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>No false positive results in circumstances of DNA damage</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>Independent of the activation of a single caspase</td>
<td>YES</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Superior sensitivity</td>
<td>YES</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Easily combined with multiple (also nuclear) immunohistochemical markers</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
</tr>
</tbody>
</table>

**Expressed Systemic Value**

- **M30** and **M65** ELISA Kit are perfectly suited for ICC of adherent cells.
- **M30** is a more sensitive compared to TUNEL.
- **M65** is perfectly suited for ICC of adherent cells.
- **M65** is easily combined with M30.

**M30-Apoptosense® ELISA Kit**

- **Annexin V**
- **TUNEL**
- **Active Caspase**

- It is not rocket science: take M30 CytoDEATH™ to obtain strong clear and reproducible staining for apoptotic epithelial cells even in archive tissue sections for retrograde studies.
- Committed to die or already dead, apoptotic cells give a sustained signal for CK18-NE recognized by M30 CytoDEATH™.
- Do you trust your TUNEL results? Double-check with M30 CytoDEATH™ and be surprised (at least by the ease of the M30 CytoDEATH™ staining protocol), which may make you switch!
- Convenience for FC and ICC: wash cells in PBS, fix in ice-cold methanol, wash/stain with M30 CytoDEATH™-FITC, wash and analyse, no special incubation buffer, no propidium iodide, no FACS signal compensation needed. Also perfectly suited for ICC of adherent cells.
To fight cancer, methods are needed that eradicate the tumor cells. Traditionally compounds are screened that are cytotoxic or inhibit cell proliferation leading to chemotherapeutic agents. Unfortunately these compounds not only kill cancerous cells, but healthy cells as well. Therefore more targeted drugs are needed which destroy cells by selectively inducing apoptosis, a form of programmed cell suicide which is morphologically and mechanistically different from necrosis, which is another form of cell death. Further profiling of drug candidates is necessary to elucidate the mechanism of action and dose response.

An alternative approach to fight cancer is the activation of the patient’s immune system to specifically detect and destroy tumor cells (immuno-therapy). The immune system uses a variety of cells and a broad arsenal of mechanisms to kill unwanted cells through induction of apoptosis. A surrogate marker to monitor the efficacy of new agents in vivo, in real time, and from early to late stages of action thus may provide the key to future success of molecularly targeted therapies.

Cytokeratin 18 (CK18) is a promising biomarker for most cancer types as they are derived from epithelial cells. CK18 is expressed in carcinomas such as lung, liver, prostate, breast and colon, whereas CK18 is absent in lymphoid, bone marrow and neuronal cells and tissues. When cells that express CK18 die through apoptosis (upon activation of effector caspase-3, -6, -7 or -9), CK18 is cleaved into proteolytic fragments liberating unaccessible epitopes (neo-epitopes; NE) at the caspase cleavage site to become an abundant and stable biomarker of apoptotic cell death that can be detected in cell supernatant or blood samples.

During apoptosis this CK18-NE-containing fragment can be detected in serum, whereas during necrosis only soluble intact CK18 released from dead cancer cells can be detected. Recent studies suggest that apoptosis is not the sole death mode of successfully targeted tumors and that it is therefore important to monitor both apoptotic and necrotic cell death during cancer treatment.

Beyond cancer research, quantification of epithelial apoptotic cell death plays an emerging role in other pathological conditions like sepsis and liver damage through infection by hepatitis virus C. Note: physiological apoptosis of epithelial cells, e.g. intestinal epithelial turnover, does not lead to significant increase of CK18-NE reactivity detectable in serum.

**AEXIS® Biochemicals** now offers a tool set manufactured by **PEVIVA** to assist apoptosis research from the first to the final step:

- **M30 CytoDEATH™ Antibody**, an established and versatile apoptosis marker which specifically detects the M30 neo-epitope of caspase-cleaved CK18 for the detection of apoptosis in epithelial tissue, including formalin-fixed archive tissue samples.

- **M30 Apoptosense® ELISA Kit** for the quantitative measurement of apoptosis in vitro and in human blood samples.

- **M65® ELISA Kit** for the quantitative measurement of total epithelial cell death (apoptosis and necrosis) in human blood samples.

**KEY LITERATURE & TESTIMONIALS**


“In conclusion, antibody M30 defines an epitope on CK18 that is independent of phosphorylation events and permits the detection of early phases of apoptosis before other methods such as the TUNEL assay or the annexin V assay.”


“Immunexpression of M30 was generally easier to interpret than ISEL, since cells giving ambiguous signals were rare with M30, but were common with ISEL.”


“We also suggest that measurement of CK18 cleavage is superior to the use of other antibodies, such as anti-cleaved poly(ADP-ribose)-polymerase, or the detection of cytochrome c release.”


“The antibody to CK18 was almost as useful and about as specific as morphologic criteria for identifying apoptotic epithelial cells. Antibodies to c-casp-3, c-lam-A, and γH2AX, though specific for apoptotic cells, were less useful. The antibody to c-PARP, though specific for apoptotic cells, had low usefulness, and the antibody to AIF was relatively nonspecific, though assessment of Apoptosis by Immunohistochemical Markers Compared to Cellular Morphology in Ex Vivo-stressed Colonic Mucosa. H. Holubeck, et al.; J Histocom. Cytohm. 53, 229 (2005)