The M30-Apoptosense® and M65® ELISA assays discriminate between different modes of epithelial cell death in vivo by quantitative measurement of either released caspase-cleaved fragments of cytokeratin 18 (ccCK18/CK18F/CK18Asp396-NE/M30) or full-length CK18 intermediate filament protein analyzed in biological fluids such as blood specimen.

The M30-Apoptosense ELISA assay utilizes the M5 capture antibody and the M30 CytoDEATH™ antibody to detect CK18 fragment containing neoepitopes (NE) at positions aa 387-396, which are generated during the early stages of apoptosis by the lethal activation of caspases-3, -7 and -9.

The M65 ELISA also detects cleaved CK18 fragments, however, it uses a different detection antibody from M30, namely M5, that does not distinguish between the full-length protein and its fragments. Thus, the M65 ELISA measures both caspase-cleavage (indicative of apoptosis) and cellular release of intact CK18 (necrosis).

Since both assays utilize the same recombinant CK18 protein fragment as a reference, the ratio of M65/M30 potentially becomes a readout, which provides additional information on the predominant mode of (tumor) cell death. These assays have been used in multiple pre-clinical and clinical research applications, but increasingly, are being used used as exploratory pharmacodynamic endpoints in clinical trials with apoptosis-modulating therapies.

During apoptosis the NE-containing CK18 fragment can be detected within stable cytokeratin complexes in serum and plasma, whereas during necrosis only soluble intact CK18 released from dead epithelial 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 specifically both apoptotic and necrotic carcinoma 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 detectable ccCK18 reactivity in serum or plasma from healthy individuals.

Caspase-cleaved Cytokeratin 18 (ccCK18/CK18F/CK18Asp396-NE/M30)
A Validated Biomarker Specific for Epithelial Apoptosis

- Selective for epithelial cells and tissues (e.g. carcinoma cell apoptosis)
- Upon its cellular release accumulates in stable protein complexes in the circulation. Generates a persistent apoptotic signature with good biostability accessible for serial blood sampling
- Convergent measure of apoptosis mediated by multiple caspases (not limited to caspase-3)
- Can be measured by standard technologies: FC, ELISA, IHC, ICC and WB
- Specific for apoptosis (detects lethal caspase activation; no false positive signal during proliferation/differentiation/inflammation)
**M30 CytoDeath™ ELISA kit**

**ALX-850-336-KI01**  
1 Kit

For the quantitative measurement of epithelial cell apoptosis *in vitro* or toxicity studies using 3D-cell culture supernatants or lysates from human organoid (e.g. hepatocytes), spheroids (cancer stem cells) or primary (tumor biopsy) tissue section cultures. Designed as a high-throughput assay for functional screening and characterization of pro-apoptotic drugs using cell culture supernatants, and spheroid or tissue lysates.

<table>
<thead>
<tr>
<th>Species Reactivity</th>
<th>Human, monkey and bovine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Type</td>
<td>Cell lysates or culture supernatants from CK18 positive (epithelial) apoptotic cells or tissues. Not suitable for serum or plasma samples.</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>60 UI, Standard Z (0 UI) + 3 S.D.</td>
</tr>
<tr>
<td>Range</td>
<td>250 – 3,000 UI</td>
</tr>
<tr>
<td>Detects</td>
<td>Soluble caspase-cleaved CK18 (ccCK18/CK18F/CK18Asp396-NE/M30)</td>
</tr>
</tbody>
</table>

**Benefits**
- Features a broader detection range specifically adapted for cellular screening systems
- Simple - no special sample/reaction buffer or standardised assay conditions required
- Flexible - accepts previously frozen cell lysates without loss of signal intensity
- Minimal hands-on time (<60 mins) with ready-to-use reagents and pre-coated ELISA plate
- Convenient 96-well microtiter plate
- Suitable for automation

**LiT:**
For a comprehensive bibliography please visit our website.

Manufactured by Peviva AB.
M30-Apoptosense® ELISA kit (CE-Mark)

ALX-850-270-KI01 1 Kit
ALX-850-270-5001 5 x 1 Kit

For the quantitative measurement of epithelial cell apoptosis in vitro and in vivo. Can be used for the quantitative and specific measurement of human tumor xenograft apoptosis in mouse models using plasma samples.

Species Reactivity Human
Sample Type Serum or plasma (EDTA, citrate, heparin plasma) samples containing caspase-cleaved CK18 from apoptotic epithelial cells. Cell lysates or culture supernatants from CK18 positive epithelial cells or tissues.
Sensitivity 25 U/l, Standard A (0 U/l) + 2 S.D.
Range 75 – 1 000 U/l

Benefits
• Quality assurance of certified (CE-marked) in vitro diagnostic (IVD) device
• Independent, robust validation (GCLP) qualifies M30-Apoptosense® ELISA as fit-for-purpose total epithelial cell death biomarker
• Two quality controls included
• Minimal hands-on time (<60 mins) with ready-to-use reagents and pre-coated ELISA plate
• Convenient 96-well microtiter plate (flexible with 12 separable 8-well strips)
• Suitable for automation

* For laboratory and research use only in USA, Canada or Japan. Not for diagnostic use in USA, Canada or Japan.

Literature References M30-Apoptosense® ELISA/M65® ELISA:

Cancer Research


Liver Disease (NAFLD/NASH/HCV/HBV/ALF/HCC) Research


Sepsis Research


For a comprehensive bibliography please visit our website.

Manufactured by Peviva AB.
M30 CytoDEATH™, mAb – The Gold Standard for the Detection of Epithelial Apoptosis

ALX-804-590-T200 Unlabeled 200 tests

Benefits

Apoptosis specific
Recognizes the CK18-ApoptD™ neo-epitope (M30) on human, monkey and bovine caspase-cleaved CK18. Does not cross-react with intact CK18 within viable cells or CK18, which is released from necrotic epithelial cells.

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 archival material, as the M30 antigen is abundant and formalin-resistant.

Flexible selection of sampling time point
Detects apoptosis earlier than Annexin V or anti-active caspase antibodies without losing signal strength at late (TUNEL-positive) stages of apoptosis.

M30 CytoDEATH™, mAb – Biotin Conjugate

ALX-804-590B-T200 Biotin 200 tests

Benefits

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 human xenograft tissue within mouse or rat samples.

M30 CytoDEATH™, mAb – Fluorochrome Conjugates

ALX-804-590F-T200 Fluorescein 200 tests
ALX-804-590OR-T200 NEW! Orange 200 tests
ALX-804-590RD-T200 NEW! Red 200 tests

Benefits

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. Can be used for multiplexing e.g. analysing circulating tumor cells (CTC) or GFP-tagged cells.

FIGURE: Detection of apoptosis in a formalin-fixed and paraffin-embedded tissue section from a human colon cancer showing confined cytoplasmic staining for tissue section from a human colon cancer formalin-fixed and paraffin-embedded.

FIGURE: HeLa cells that received combined treatment with CHX and TRAIL show a sustained and strong caspase activity and concomitant cCK18/cCK18F accumulation as detected by the M30 CytoDEATH™ Fluorescein (Prod. No. ALX-804-590F) antibody.

FIGURE: HeLa (human cervical cancer) cells were fixed in methanol and stained with M30 CytoDEATH™ Orange antibody (Prod. No. ALX-804-590OR). Blue line: Apoptotic HeLa cells (preincubated with CHX-10ug/ml for 1h- followed by rhsTRAIL-200ng/ml for 24h) were stained with M30 CytoDEATH™ Orange. Green line: Untreated, viable HeLa cells stained with M30 CytoDEATH™ Orange. Red line: Untreated, viable HeLa cells were left unstained.

Related Products

<table>
<thead>
<tr>
<th>Product</th>
<th>Isotype</th>
<th>Specificity</th>
<th>Application</th>
<th>Prod. No.</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokeratin 18 (human), mAb (M5)</td>
<td>Mouse IgG2b</td>
<td>Human</td>
<td>FC, ICC, IHC (FS, PS), IP, WB</td>
<td>ALX-804-640-T200</td>
<td>200 tests</td>
</tr>
<tr>
<td>Cytokeratin 18, mAb (M6)</td>
<td>Mouse IgG2a</td>
<td>Human, mouse, rat and dog</td>
<td>FC, ICC, IHC (FS, PS), IP, WB</td>
<td>ALX-804-641-T200</td>
<td>200 tests</td>
</tr>
</tbody>
</table>