Oncology Cell Death Products

from basic research to disease modelling to clinical trials
Peviva Cell Death Products

Monoclonal Antibody Products

- M5 and M6 Keratin 18 mAb
- M30 CytoDeath™ caspase-cleaved K18 mAb (unlabelled, biotin, fluorescein, orange)

Sample Type & Suitable Experiments

<table>
<thead>
<tr>
<th>Product</th>
<th>Cell cultures</th>
<th>Spheroids</th>
<th>Xenografts</th>
<th>Blood samples</th>
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<tbody>
<tr>
<td>M30 Apoptosense ELISA</td>
<td>(✓)</td>
<td>(✓)</td>
<td>✓</td>
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<tr>
<td>M30 CytoDeath ELISA</td>
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<td>M65 ELISA</td>
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<td>M65 EpiDeath ELISA</td>
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Peviva Cell Death ELISAs

Measurement of Cell Death Modes

<table>
<thead>
<tr>
<th>Product</th>
<th>Apoptosis</th>
<th>Necrosis</th>
<th>Total cell death</th>
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The Peviva ELISAs are

- a tool for determination of cell death mode
- for cell culture/spheroids OR *in vitro* serum/plasma samples
Keratin 18 in the Human Body

**Keratin 18** is found in almost all **epithelial** cells in the body, e.g.:
- liver
- lung
- intestines
- breast
- prostate

and **tumors** of these organs.

K18 is **not** expressed by neurons, muscle and connective tissues, skin and cells of the immune system.
Biology and Mechanisms of Epithelial Cell Death:

Necrosis vs. Apoptosis
Epithelial Cell Death: **Necrosis or Apoptosis**

**Necrosis**
- Only intact K18
- Leakage of full-length K18

**Apoptosis**
- Caspase-cleaved K18
- Disintegration of apoptotic bodies and release of K18 fragments
- Asp396-Neoepitope

**M65 EpiDeath® ELISA**
- M65® ELISA
- Measurement of intact and cleaved K18
- M65 EpiDeath® ELISA measures total cell death (necrosis and apoptosis)

**M30 CytoDeath™ ELISA**
- M30 Apoptosense® ELISA
- Measurement of cleaved K18 only
- M30 Apoptosense® ELISA measures only apoptosis
Oncology Drug Development Applications
1) Determine Drug Mechanism of Action

M30 Apoptosense® ELISA + M65® ELISA: Identify Mechanism of Cell Death in Antitumor Drugs

- **M30**: detects *caspase-cleaved* cytokeratin 18 = APOPTOSIS
- **M65**: detects *intact* cytokeratin 18 = NECROSIS

M30:M65 ratios are used to determine cell death mode (apoptosis vs. necrosis):

- **Apoptosis**
  - M30 level
  - M65 level
  - High levels of caspase-cleaved K18 (ccK18) compared to total K18 (high M30:M65 ratio)

- **Necrosis**
  - M30 level
  - M65 level
  - Low levels of caspase-cleaved K18 (ccK18) compared to total K18 (low M30:M65 ratio)

2) Determine Drug Mechanism of Action

Cancer Therapeutics Often Induce Apoptosis

- M30 antibody detects apoptosis with high specificity.
- Apoptosis from only K18 positive cells, such as epithelial tumour cells, is detected.

(K18 negative) fibroblasts treated with paclitaxel only induce M30 signal increase when transfected with K18 cDNA.
Measure Apoptosis Independently from Time

The accumulated apoptosis product measured by the M30 CytoDeath™ ELISA:

Approach combines high-throughput & end-point measurements. Assay quantifies accumulation of an apoptosis-generated product, which is semi-stable in cells and culture media. The signal from cells that have undergone apoptosis remains in the culture medium (or blood) after all apoptotic processes are completed and all cells are dead. A late time point ($t_3$ in the chart below) is sufficient to quantify the intensity of the apoptotic stimulus, regardless of its kinetics.

Methods that measure the number of apoptotic cells at $t_1$ or $t_2$ will often return incorrect results.
3) Determine Drug Mechanism of Action

Data in the graphs above indicate screening compound NSC567461 triggers an apoptotic response in tumor cells at low concentrations and a necrotic response at higher concentrations.

*Erdal et al., PNAS (2005) 192-197.*
4) Drug Screening: High Through-Put Applications

M30 CytoDeath™ ELISA:
Identify Pro-Apoptotic Antitumor Drugs via High-Through-Put-Screening

1. Grow **human epithelial** tumor cell lines in culture.
2. Test drug candidates/compound library on cell lines to determine effective **pro-apoptotic** compound(s).

### Tumor Cell Lines (ex. NCI60)

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<th>PLATE</th>
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<th>MEASURED NO</th>
<th>SAMPLE</th>
<th>SPONCR/CONV/CONV/VOLN/LEVEL/TH</th>
<th>SHAR</th>
<th>SOURC</th>
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### Compound Screening Library

National Cancer Institute (NCI)/Division of Cancer Treatment and Diagnosis (DCTD)/Developmental Therapeutics Program (DTP) - [http://dtp.cancer.gov](http://dtp.cancer.gov)
5) Preclinical Drug Testing in Disease Models

M30 CytoDeath™/M30 Apoptosense® ELISAs: Evaluate Efficacy of Pro-Apoptotic Antitumor Drugs

2D Cell Culture or 3D Spheroids
M30 CytoDeath™ ELISA
M65® ELISA (necrosis assessment)

Xenograft Models
M30 Apoptosense® ELISA
M65® ELISA (necrosis assessment)

- Minimal/no antibody cross-reactivity with mouse & rat species
- Apoptosis in tumor xenograft leads to increase in human ccK18 in mouse plasma

6) Drug Testing in Oncology Research Studies

• Data from a research study where ccCK18 was induced during each cycle of chemotherapy is shown above (right). In the graphs, estramustine phosphate (EMP) in combination with docetaxel did not show a treatment effect (shown by stable ccCK18 serum levels and increasing PSA concentrations (left)). Switching to EMP-docetaxel therapy led to increases of ccCK18 levels in serum which correlated with decreases of PSA.

• Typically, samples should be tested 2-4 days after drug administration, depending on pharmacokinetics and mechanism of action.  

_Kramer et al., BJC 94 (2006) 1592-1598._
7) Drug Toxicity Evaluation in Oncology Research Studies

- Use in drug safety research for non-epithelial tumors to assess treatment toxicity.

- CK18 levels as measured by the M65 ELISA® measure host toxicity; rises in CK18 levels provide early warning of epithelial toxicity.

Changes in circulating CK18 in research participants with lymphoma following chemotherapy, according to CTCAE epithelial toxicity score.