

# Apoptosis detection in cell cultures

## M30 CytoDeath™ ELISA

The M30 CytoDeath™ ELISA specifically detects a caspase-cleaved cytokeratin-18 fragment. The M30 CytoDeath™ ELISA can be used to quantify apoptosis of epithelial cells in cell culture experiments. The assay has a dynamic range and sensitivity suitable for in vitro work. The M30 CytoDeath™ ELISA is a powerful drug screening tool and is useful for in vitro characterization of apoptosis-inducing drugs.

### Applications

- Determining **apoptosis in cultured epithelial cells** (not suitable for serum/plasma). Examination of **time-kinetics** and **dose-response** relationships.
- Determining apoptosis in multicellular **spheroids** and **organ cultures**.
- Studies of the **mechanisms of action** of drugs that induce apoptosis (using siRNA and inhibitors).
- A useful tool for **drug screening**.
- Determining death mode (**apoptosis or necrosis**).
- Can be used for **human, monkey and bovine cell lines** (not mouse, rat and canine).



### Features

The M30 CytoDeath™ ELISA has the following advantages:

- **High-throughput:** The assay is in the 96 well format.
- **Sensitivity:** Apoptosis is easily detected in cultures containing 2,000 cells (10,000 recommended).
- **End-point assay:** The assay measures the accumulation of a caspase-cleaved protein (CK18) in cells and culture medium over time. The total levels of caspase-cleaved CK18 protein fragments are determined after adding non-ionic detergent to tissue culture medium.
- **Measuring an endogenous cleavage product:** In addition to standard monolayer cultures, the assay can be used for multicellular spheroids and for organ cultures.
- **Easy to use:** A minimal number of steps are required. Results are recorded as absorbance at 450 nm using standard 96 well plate readers.
- **Reagents are ready-to-use and have a long shelf life at +4 °C.**

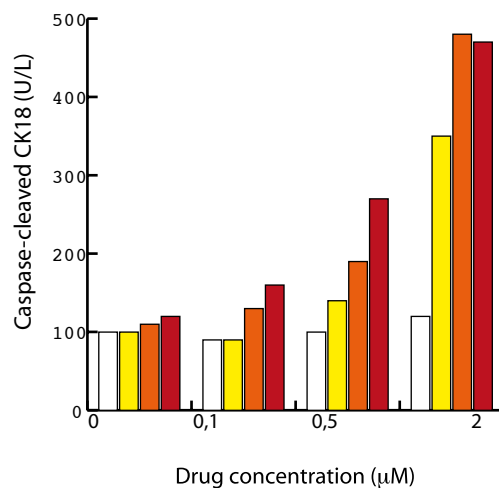
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## Apoptosis Detection in Cell Cultures

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Initial studies of stimuli that induce apoptosis (e.g. using different drugs) need to focus on the dose-response and the time-course of apoptosis induction. Such studies are conveniently performed using the M30 CytoDeath™ ELISA. Cells grown in 96 well plates are exposed to drugs. An aliquot (cells and medium) from each well is then transferred to the M30 CytoDeath™ ELISA plate for determination of the caspase cleaved CK18 product. The assay is specific for **epithelial cells** expressing CK18.

The M30 CytoDeath™ ELISA is an **end-point assay**. The assay measures the **accumulation** of caspase-cleaved CK18 in cells and culture medium over time. The total amount of caspase-cleaved CK18 protein is measured after adding non-ionic detergent to tissue culture medium (Figure 1).



**Figure 1**

**Detection of apoptosis. Human MDA-MB-231 breast cancer cells were seeded at 10,000 cells per well in a 96-well plate. The next day, cells were treated with different concentrations of an apoptosis-inducing substance. The agent was added at different times to achieve the desired exposure times as indicated. At the end of the exposure period, non-ionic detergent was added to the medium and the plate was frozen at -20°C.**

**After thawing the plate at room temperature, 25 µl was transferred from each well to an M30 CytoDeath™ ELISA plate. The result was obtained after 4 hours and 30 minutes (including a 4 hours incubation). The procedure involved only 8 pipetting steps (including 5 washing steps).**

Reference: Hägg et al., *Mol Cancer Therapeutics* 3, 489, 2004.

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## Measuring Apoptosis in Multicellular Spheroids

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Investigation of the mechanism of action of anticancer drugs is typically performed using monolayer cultures of tumor cells. It is well established that such monolayer cultures do not represent the characteristics of three-dimensional solid tumors. The multicellular tumor spheroid model is of intermediate complexity between in vivo tumors and in vitro monolayer cultures and is more suitable than monolayers for various studies of anticancer drugs.

The M30 CytoDeath™ ELISA method is a versatile tool to examine apoptosis in **multicellular spheroids**. It will measure the formation of caspase-cleaved cleavage product in the interior of spheroids. The method does not require exogenous substrates that do not freely diffuse into multicellular spheroids.

It is also possible to use **tissue slices from ex vivo tumors** to measure apoptosis. In this application, tumors are sliced and explanted into culture. Drugs are added and the levels of cleaved product released to the medium is analysed. The **epithelial cell specificity** of the M30 CellDeath™ ELISA is a major advantage: apoptosis of stromal cells will not be detected since these cells do not express CK18.



References: Herrmann et al., *J. Biomol. Screening* 13: 1, 2008;  
Hägg Olofsson et al., *Clin Cancer Res.* 13: 3198, 2007.

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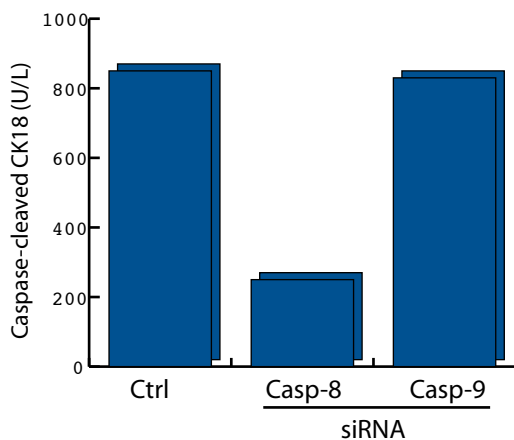
## Studies of the Mechanisms of Apoptosis Induction

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It is of vital importance to understand the mechanism(s) of an apoptotic stimulus. Is the extrinsic or intrinsic pathway involved? Which signaling pathways are required for apoptosis?

The M30 CytoDeath™ ELISA is a convenient tool for such mechanistic studies. The assay allows rapid evaluation of whether specific gene products/enzymes are required for apoptosis induction.

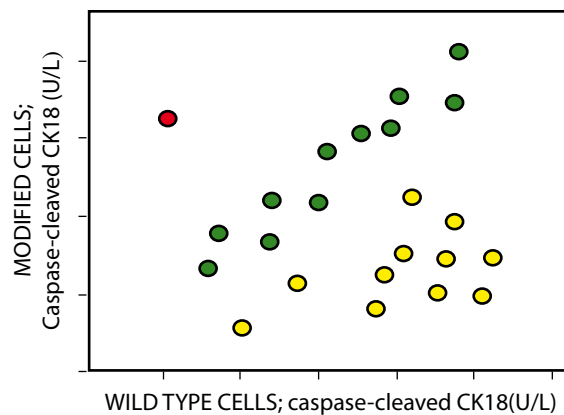
- Cells can be treated with apoptotic stimuli in the presence and absence of pharmacological inhibitors. The effects of target inhibition (caspases and other proteases, protein kinases etc.) on apoptosis is then easily evaluated.
- Cells can be treated with apoptotic stimuli in the presence and absence of siRNA to down-regulate specific genes (Figure 2).
- Pairs of cell lines with different phenotypes can be treated with the same apoptotic stimuli. Ideally, such pairs will differ with respect to a single property, e.g. the p53 tumor suppressor (Figure 3).



**Figure 2**

*siRNA was used to knock down caspase-8 or caspase-9 expression prior to treatment of HCT116 cells with TRAIL. Cells were transfected with siRNA and incubated for 48 hours. TRAIL was then added and apoptosis was measured after 24 hours using the M30 CytoDeath™ ELISA. Note the inhibition of induction of caspase-cleaved CK18 after knock-down of caspase-8, but not of caspase-9.*

References: Hägg et al., *Molecular Cancer Therapeutics* 3, 489, 2004; Erdal et al., *Proc Natl Acad Sci USA* 102, 192, 2005; Berndtsson et al., *Int J Cancer* 120: 175, 2007.



**Figure 3**

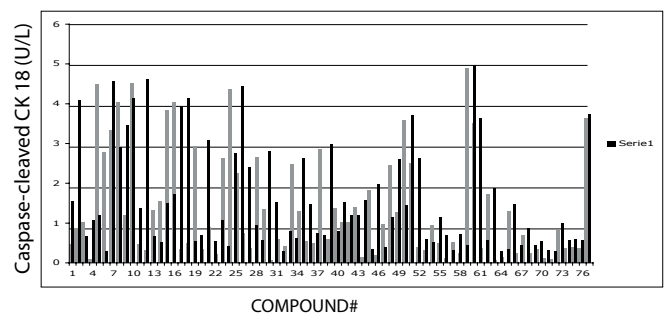
*Human carcinoma cell lines differing with respect to the status of specific gene are treated with apoptosis-inducing drugs. Apoptosis is measured after 24 hours using the M30 CytoDeath™ ELISA. Most drugs induce the same apoptotic response in both cell types (green symbols). Some drugs induce a stronger response in cells with the wild type phenotype (yellow symbols); one drug induces a stronger response in the knock-out cells (red symbol) (see Erdal et al, 2005).*

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## Drug Screening

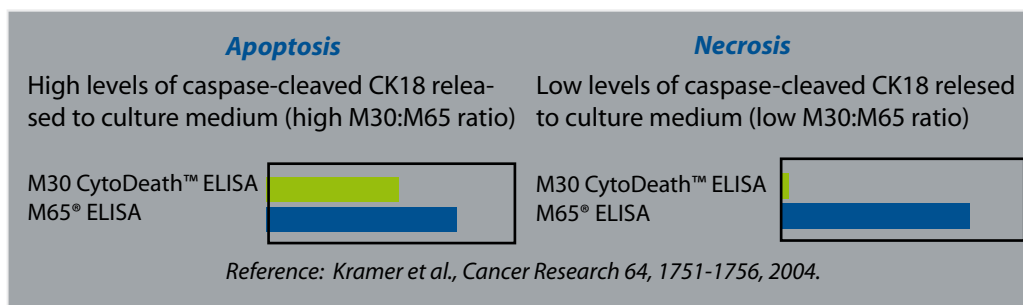
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The M30 CytoDeath™ ELISA is useful for drug screening/chemical biology. A major advantage of the assay is that it measures accumulation of the apoptosis product. Apoptosis will therefore be detected also at late time-points when all cells in the culture are dead. This reduces the complexity of screening experiments.



## Determination of Cell Death Mode

The PEVIVA products can be used to conveniently determine whether a particular stimulus induces **apoptosis** or **necrosis**. This is done by measuring the release of caspase-cleaved and total CK18 into the cell culture medium. Total CK18 is measured using PEVIVA's M65<sup>®</sup> ELISA<sup>®</sup> assay (prod. no. 10020).



## Tool Kit for Translational Research

The PEVIVA products represent powerful tools for translational research. Apoptosis can be studied in both **in vitro** experimental settings and **in vivo** using plasma or blood from experimental animals and patients:

### M30 CytoDeath™ ELISA

**In vitro studies** of monolayer cell cultures, multicellular spheroids and organ cultures

### M30-Apoptosense<sup>®</sup> ELISA

**Serum/plasma biomarker studies:** in vivo studies of human cancer and liver disease. Useful tool for studying response of human tumor xenografts in mice to apoptotic stimuli

### M65<sup>®</sup> ELISA

**Serum/plasma biomarker studies** and **in vitro** studies of total epithelial cell death.

## HOW TO ORDER

**Products can be ordered from our local distributors, or, directly from PEVIVA for worldwide deliveries. Please consult [www.peviva.se](http://www.peviva.se) for further details on pricing, how to order and for shipment information.**



PEVIVA AB Strömkarlsvägen 82, SE-167 62 Bromma/Sweden  
Website: [www.peviva.se](http://www.peviva.se) E-mail: [info@peviva.se](mailto:info@peviva.se)  
Telephone: + 46 (0)8 80 88 83 Telefax: + 46 (0)8 564 626 36

