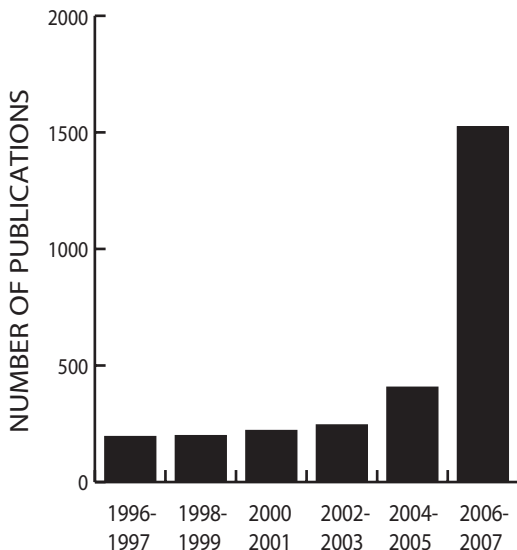


Multicellular tumor spheroids represent models for the three-dimensional microenvironment of tumors. Spheroids are superior to monolayer cultures for applications such as anticancer drug research. Apoptosis of tumor cells in multicellular spheroids can be quantified using the **M30 CytoDeath™ ELISA**.



APOPTOSIS *in* DETECTION MULTICELLULAR SPHEROIDS

Publications containing "tumor spheroids" on PubMed



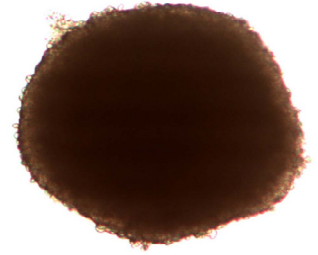
The interest of the scientific community to use **multicellular spheroids** as *in vitro* models of tumors has increased in recent years. Multicellular spheroids are known to better mimic the 3D conditions of tumors compared to monolayers cultures. The deeper spheroid cell layers contain non-proliferating cell populations that respond poorly to radiation and chemotherapy.

Simple procedures to determine cell proliferation and cell death in spheroids have been lacking. Spheroids are compact and difficult to dissociate into single cell suspensions. Exogenous substrates to measure cell viability may not penetrate into spheroids.

The M30 CytoDeath™ ELISA allows the quantitation of an endogenous apoptosis product (caspase-cleaved cytokeratin 18) using spheroid lysates.

Multicellular spheroids as models for 3D tumor tissue

Two-dimensional (2D) monolayer cultures are universally used in experimental studies in biology. Monolayer cultures are convenient to use but do not provide accurate models for the microenvironment of tumors. Tumor cells exist in an organized 3D matrix where they are surrounded by other cells such as fibroblasts, macrophages and lymphocytes. Whereas nutrients and oxygen will be easily accessible to all tumor cells in 2D culture, this is not the situation in 3D tumor tissue. A large number of studies have reported differences in cell proliferation and phenotypic properties between cells grown in 2D or 3D culture. It is therefore advisable to use multicellular spheroids in various projects.



A HCT-116 colon cancer spheroid after 7 days of incubation.

M30 CytoDeath™ ELISA: Main features

- *Quantification of apoptosis of epithelially derived cells (spheroids, organ cultures, monolayer cultures)*
- *Easy to use, 6-step quantification*
- *Reproducible and reliable measurements*
- *Standard microplate reader sufficient (A450)*
- *End-point measurement*

Key requisites:

- epithelium-derived cell lines
- cells expressing CK18
- human, monkey or cow cells
- does not detect CK18 from rodents

6-step apoptosis quantification

1. **Transfer 25 µl sample to the microtiter plate**
2. **Add 75 µl diluted conjugate and incubate for 4 hours**
3. **Wash**
4. **Add 200 µl TMB and incubate for 20 minutes**
5. **Add 50 µl Stop solution**
6. **Read absorbance at 450 nm**

Determination of apoptosis by the M30 CytoDeath™ ELISA method

The M30 CytoDeath™ ELISA quantifies apoptosis by measuring a neo-epitope generated by caspase cleavage of cytokeratin 18.

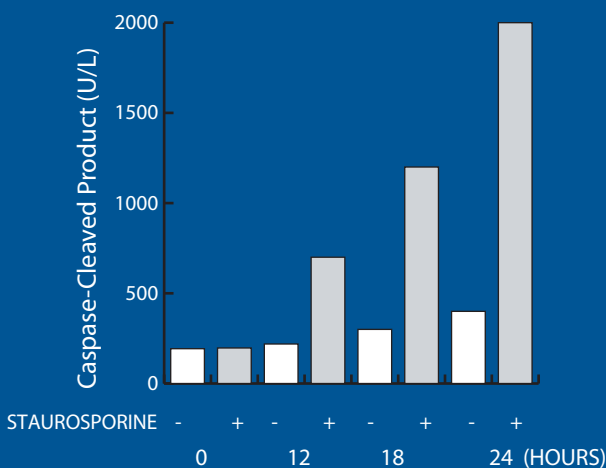
The caspase cleaved apoptosis products will be present in apoptotic cells and will also be released from dying cells into the cell culture. The total content of the apoptosis product in spheroid cultures is measured after addition of non-ionic detergent (NP-40) to the culture medium. The M30 CytoDeath™ ELISA method therefore provides an **integrative measure of apoptosis in spheroid cultures.**

In vitro culture of multicellular spheroids

It is possible to generate spheroids of homogeneous size using the “**hanging drop method**” (Kelm *et al.*, 2003). A spheroid diameter of 500 μm is reached after 7 days of incubation. Spheroids will contain proliferating cells in the outer layers, and quiescent, necrotic and apoptotic cells in the central portions. The possibility to generate one 500 μm spheroid per well in 96-well microtiter plate is very attractive in various biological studies, particularly in studies of the response of tumor cells to drugs.

It is possible to use spheroids for **drug screening**. Herrmann *et al.* (2008) have demonstrated excellent performance characteristics of a screening assay based on colon carcinoma spheroids and measurement of caspase-cleaved cytokeratin 18 (Z' values of > 0.5). The M30 CytoDeath™ ELISA method provides the opportunity to restrict the number of primary hits in cell-based screening campaigns to compounds with good tumor penetration.

Increase of caspase-cleaved CK18 in spheroids



Measurement of apoptosis using the M30 CytoDeath™ ELISA:

Apoptosis was induced by treatment with 0.5 μM staurosporine in spheroids supernatant. The spheroids were grown by the hanging drop method as described above and treated at the 7th day of growth; size 500 μm in diameter. Comparison with untreated control spheroids.

Technical notes:

- The M30 CytoDeath™ ELISA can only be used for human, monkey or cow epithelial cell lines expressing cytokeratin 18. The cells must be capable of spheroid formation (aggregation and compaction). Fibroblasts can be mixed in to mimic in vivo conditions (fibroblasts do not express cytokeratins and will not contribute to the apoptosis signal).
- A cell suspension of 50 000 cells/mL is pipetted in the rings of the lid of a 96 well plate (each drop consisting of 20 μl). The lid is placed upside down and stored in a cell culture incubator with the drops hanging from the lid. Within some hours, the cells in the drop aggregate to a small spheroid.
- At the third day, the spheroids can be centrifuged down to the wells of the 96 well plate (3 minutes at 1 000 rpm)
- After 5 – 7 days of incubation, the spheroids in the drop have grown to 500 μm in diameter and can be used for experiments (e. g. treated with drugs, cytokines or other agents).
- After treatment, spheroids are lysed by adding non-ionic detergent to the medium (1% NP-40).
- Caspase-cleaved cytokeratin 18 is measured using the M30 CytoDeath™ ELISA.

References

The Products from PEVIVA have been used by 10 of the 20 biggest pharma companies in the world. A variety of published articles are available presenting research done with PEVIVA's products or the validation of the assays.

Please consult www.peviva.se for further references.

Original article

Leers, M.P. *et al*, Immunocytochemical detection and mapping of a Cytokeratin 18 neo-epitope exposed during early apoptosis. *J Pathol* 187, 567-572 (1999)

Spheroid article

Herrmann, R. *et al*, Screening for Compounds that Induce Apoptosis of Cancer Cells Grown as Multicellular Spheroids. *J Biomol Screen*. 2008 Feb;13(1):1-8. (Epub 2007 Nov 26.)

M30 related articles

Kelm *et al*, Screening for Compounds that Induce Apoptosis of Cancer Cells Grown as Multicellular Spheroids. *J Biomol Screen*. 2008 Feb;13(1):1-8. (Epub)

Hägg, M. *et al*, A novel high-through-put assay for screening of pro-apoptotic drugs. *Invest. New Drugs*, 20: 253-259 (2002)

Erdal, H. *et al*, Induction of lysosomal membrane permeabilization by compounds that activate p53-independent apoptosis. *Proc. Natl. Acad. Sci. USA* 102, 192-197.

Other products from PEVIVA

M30 Apoptosense® ELISA

Prod. no. 10010

M65® ELISA

Prod. no. 10020

M30 CytoDeath™ ELISA

Prod. no. 10900

M5 CytoKERATIN™ antibody

Prod. no 10600

M6 CytoKERATIN™ antibody

Prod. no 10650

M30 CytoDEATH™ antibody

Unconjugated: prod. no 10700

Biotin: prod. no 10750

Fluorescein: prod. no 10800



M30 CytoDeath™ ELISA kit components

- M6 coated microtiter plate with 96 wells
- M30-HRP Conjugate (400 µL)
- Conjugate Dilution Buffer (11 mL)
- 4 Standard Points (Zero, Low, Medium, High, 500 µL each)
- TMB Substrate (22 mL)
- Stop Solution (6 mL)
- Wash Tablet
- Instructions for use
- List of components



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