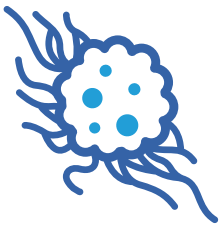
PEVIVA

diapharma®

The PEVIVA Products in Oncology Research



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The Use of the PEVIVA Products in Oncology Research

The PEVIVA product line is manufactured by VLVbio, a Swedish biotechnology company devoted to the development, manufacture and sale of unique human specific biomarker assays and antibodies intended for

- preclinical research on anti-cancer drug development
- rapid and non-invasive measurement of anti-cancer drug efficacy in research studies

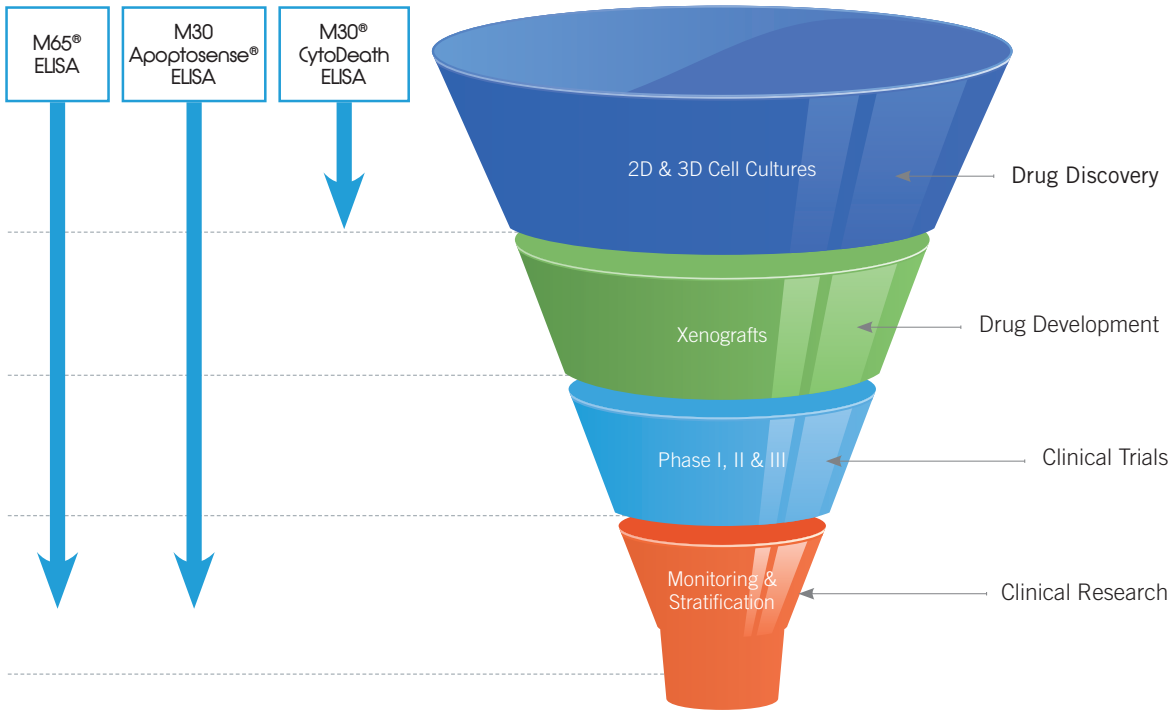
The M30 Apoptosense® ELISA (M30®) and the M30 CytoDeath™ ELISA (M30®) detect caspase-cleaved keratin 18 (ccK18) and are sensitive and specific biomarker assays for the measurement of apoptosis.

The M65® ELISA detects both caspase-cleaved and intact keratin 18 (K18). It is therefore a biomarker assay for the measurement of total cell death due to necrosis and apoptosis.

The PEVIVA products are valuable in all of the stages in anti-cancer drug development, presented in detail in the following sections

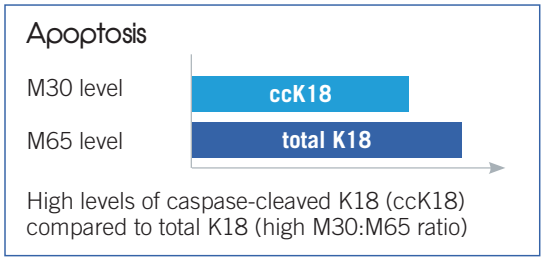
- ✔ **DRUG DISCOVERY,** where potential drug candidates are selected and tested in vitro for their basic chemical, physical and biological properties
- ✔ **DRUG DEVELOPMENT,** where the compound is refined and tested in animal models for efficacy and toxicity
- ✔ **CLINIC TRIALS,** where compounds are tested for toxicity and efficacy
- ✔ **CLINICAL RESEARCH,** where the effect of a potentially new therapy can be seen during the treatment course

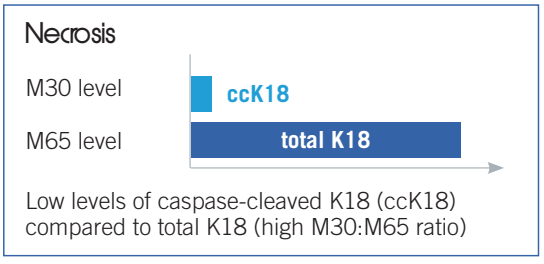
The PEVIVA Products in Anti-Cancer Drug Development



The M30® ELISAs and the M65® ELISA can be used on samples from cell cultures and spheroids, human serum and on samples from mice carrying human xenografts.

Product	Apoptosis	Total Cell Death	Cell Cultures	Spheroids	Xenografts	Blood/Plasma Samples
M30 Apoptosense® ELISA	✔	✖	✔	✔	✔	✔
M30 CytoDeath™ ELISA	✔	✖	✔	✔	✖	✖
M65® ELISA	✖	✔	✔	✔	✔	✔





The ratio between the M30 Apoptosense® and the M65® ELISA reflect the cell death mode. The amount of apoptosis (M30) is compared to the amount of total cell death (M65) by calculating the M30:M65 ratio. High M30:M65 ratios indicate that the cell death is mainly due to apoptosis. In contrast, low M30:M65 ratios suggest necrosis is the predominant cause of cell death.

M30® ELISAs and M65® ELISA in Drug Discovery

Screening of Novel Anticancer Agents in 2D Cultures

A simple cost-effective means of studying the efficacy of new anti-cancer compounds are two-dimensional (2D) cell cultures. By measuring the mode of cell death (necrosis and/or apoptosis) with the M30® ELISAs and the M65® ELISA, estimations of the efficacy of a compound can be obtained.

The M30® ELISAs and M65® ELISA in cancer studies of 2D cultures

- ✔ The M30® ELISAs can specifically identify compounds that induce apoptosis, a major target of anti-cancer drugs
- ✔ The M30® ELISAs and the M65® ELISA describe the relative contribution of apoptosis and necrosis
- ✔ The M30® ELISAs and the M65® ELISA only require measurements at a single, specific timepoint, as they measure the accumulation of intact and cleaved K18
- ✔ The M30® ELISAs and the M65® ELISA can also be used when examining time-kinetics and dose-response relationships

EXAMPLE
Researchers used the M30 CytoDeath™ ELISA to screen for compounds with anti-tumor effects on 2D cell cultures.

Findings

By using the M30 CytoDeath™ ELISA, 40 compounds that induced apoptosis (hits) were identified from a library of 999 compounds.

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The M30® ELISAs provide valuable information in 3D cell cultures, and can help in selecting potential drug candidates to investigate further.

Screening of Novel Anticancer Agents in 3D Cultures

In drug discovery, "hits" need to be further studied in order to be developed into leads and then candidates. Three-dimensional (3D) cell cultures are yet another effective method for preforming such studies. 3D cultures can be produced by culturing cells in hanging drops over microtiter wells, forming spheroids.

The M30® ELISAs in cancer studies of 3D cultures

- ✔ The M30® ELISAs are K18 specific and useful in co-cultures, as they only detect apoptosis in K18 positive cells (simple epithelial cells)
- ✔ The M30® ELISAs enable accurate identification of pro-apoptotic compounds
- ✔ The M30® ELISAs only require measurements at a single, specific timepoint, as they measure the accumulation of intact and cleaved K18

EXAMPLE 1

Researchers used the M30 CytoDeath™ ELISA on 3D cell cultures and generated 40 lead compounds from the 382 hits identified in a preliminary 2D screen. These 40 leads were re-tested at a lower concentration and found that 11 of these 40 compounds still showed activity.

The method does not require single, specific time points of drug incubation. This is in contrast to many cellular apoptosis assays, which must be performed at time points when apoptotic cells maintain membrane integrity.

EXAMPLE 2

Researchers present the basic techniques for developing spheroidal cultures and their use for studying the pro-apoptotic effects of drugs. The researchers showed that the M30 Apoptosense® ELISA could be used to study the development of apoptosis and during screening of compounds in spheroids.

Findings

By using the M30 Apoptosense® ELISA, different drugs could be shown to cause cell death by different mechanisms. For example, an antifungal medication induces apoptosis more strongly in spherical cultured compared with monolayer cultures, whereas a chemotherapy agent induces stronger apoptosis in monolayer cultures than in spheroids.

M30 Apoptosense® ELISA and M65® ELISA in Drug Development

Studying Cell Death and Drug Efficacy in Rodent Xenograft Models

Human tumour xenografts are widely used as pre-clinical models for anti-cancer drug efficacy in humans. The M30 Apoptosense® ELISA and the M65® ELISA are human specific and thus only measure tumour cell death and not rodent liver toxicity in xenograft models, making them ideal tools for studies on Pharmacodynamics and Pharmacokinetics.

The M30 Apoptosense® ELISA and the M65® ELISA in cancer studies using xenograft models:

- ✔ The M30 Apoptosense® ELISA and the M65® ELISA can determine tumour response in blood from xenografts, and further on in patients, making it a powerful tool for translational studies of anti-cancer drugs
- ✔ The M30 Apoptosense® ELISA and the M65® ELISA measure only increases of human K18 in the blood of the rodent, as they do not cross-react with rodent proteins
- ✔ The M30 Apoptosense® ELISA and the M65® ELISA are used to investigate the dose and time response of potential drug candidates prior to human trials

EXAMPLE 1
Researchers studied mice inoculated with a human head-neck carcinoma cell line and rats with colon cancer cells. The researchers demonstrated that the release of K18 fragments from xenografts in mice and rats treated with the anti-cancer drug could be measured with the M30 Apoptosense® ELISA.

Researchers concluded that a dose response relationship of CK18-Asp396 release can be established in xenograft models.

These findings suggest that CK18 blood markers will be useful for PK/PD studies, information which will be useful in subsequent clinical studies.

The possibility to use the M30 Apoptosense® ELISA assay to determination of tumor response in blood from both xenograft models and from subjects provides a powerful tool for translational studies of anticancer drugs.

EXAMPLE 2
Researchers studied an experimental drug in xenografts. They showed that it caused a decrease in metastatic colony formation and that this was associated with increased levels of total K18 in serum, measured with the M65® ELISA. The researchers also showed that increases in serum K18 levels were related to therapy response.

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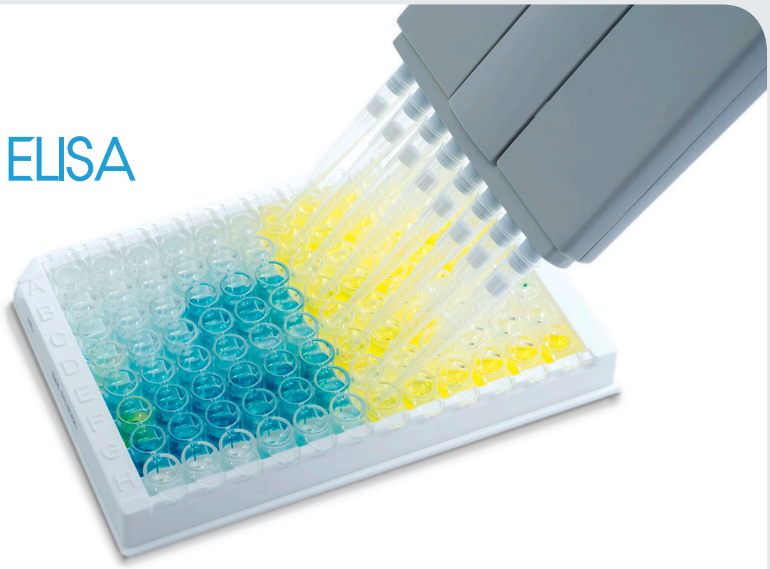
M30 Apoptosense® ELISA and M65® ELISA in Clinical Trials



Investigation of Drug Candidates in Clinical Trials

In clinical trials, candidate drugs are administered to patients to investigate whether the substance induced cell death in tumours with sufficient efficacy.

The M30 Apoptosense® ELISA and the M65® ELISA assays in clinical trials:

- ✔ When monitoring the kinetics of cell death during therapy, the M30 Apoptosense® ELISA and the M65® ELISA provide valuable information about the effect of the therapy
- ✔ Increases in K18 fragments and total K18 during therapy indicate a therapeutic response

EXAMPLE 1
Researchers studied the effect of a compound on lung cancer subjects. They found that by using the M30 Apoptosense® ELISA and the M65® ELISA assays, they could follow the time course, efficacy and investigate the cell death mechanism. The researchers found that by using the M30 Apoptosense® ELISA and the M65® ELISA assays, they were able to show the time course and efficacy of the agent as well as investigate its cell death mechanism.

The study represents one of the first showing that M65 ELISA measurements can potentially discriminate between different response groups during a phase I trial of a novel anticancer agent.

The increase in M65 concentrations detected 24 h after administration of the compound was associated predominately (and significantly) with subjects who exhibited stable disease or partial response to the drug.

Findings

The M30 Apoptosense® ELISA and the M65® ELISA could detect a response after only 24–48 hours and the time course of the response was shown. Subjects with early progression had higher M65® ELISA levels prior to therapy. Subjects with stable disease showed the greatest increase in total K18 during therapy, measured with the M65® ELISA.

EXAMPLE 2
Researchers evaluated the use of the M30 Apoptosense® ELISA and the M65® ELISA to study the effect of a drug candidate in a phase I clinical trial.

Therapy response was monitored by measuring serum total K18 and K18 fragments and the immunohistological proliferation biomarkers. In subjects who received the highest doses of the compound, decreases in the proliferation biomarkers were associated with increases in total K18 and K18 fragments in serum.

M30 Apoptosense® ELISA and M65® ELISA in Clinical Research

Cell Death Response Measurement

By using the M30 Apoptosense® ELISA and the M65® ELISA the efficacy of potential new therapies can be demonstrated during the course of treatment. In contrast to other techniques or biomakers, the rate of cell death is an immediate response of successful therapy indicated within hours or days from induction of potential drug candidate.

The M30 Apoptosense® ELISA and the M65® ELISA are valuable for measuring the effects of new potential anti-cancer drug candidates:

- ✔ Short time elevations in K18 fragments (M30) and total K18 (M65) may be evaluated
- ✔ Persistently elevated K18 fragments (M30) and total K18 (M65) can be measured
- ✔ Relative concentrations of K18 fragments (M30) and total K18 (M65) provide information regarding to the mechanism of the drug, i.e. apoptosis or necrosis

EXAMPLE 1
Researchers used the M30 Apoptosense® ELISA and the M65® ELISA assays to compare changes in serum K18 fragments and total K18 with the traditional biomarker PSA in subjects with prostate cancer. Different patterns of increases in total K18 and K18 fragment level were observed, which correlated to drug regime and subject response. Repeated, temporary elevations of serum K18 fragment concentrations in response to therapy were associated with a decrease in PSA levels and a shirnkage of tumor size (by imaging technique) over the course of treatment.

Findings

Measurements of caspase-cleaved K18 (M30) in serum can clarify the relative effectiveness of different treatment modalities.

EXAMPLE 2
Researchers performed a similar study on breast cancer subjects. This study demonstrated that a therapeutic response was associated with the release of total K18 and K18 fragments as measured by the M30 Apoptosense® ELISA and the M65® ELISA. Similarly, a chemotherapy medication caused increases in both M65 and M30 levels, but CEF therapy resulted predominantly in the release of total K18. This indicates that CEF predominantly induces necrosis in tumor cells.

Researchers conclude that serum CK18 measurements may be useful for assessing treatment effects. The data suggesting that the initial cell death response determined by CK18 biomarkers is an important determinant of treatment outcome. The method is robust and samples can be frozen and stored before analysis, making the method suitable for multicenter clinical trials of novel anticancer drugs.

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Stratification of Study Subjects

The M30 Apoptosense® ELISA and the M65® ELISA can be useful tools when researching progression of disease and prognosis among study subjects, and when discriminating between healthy individuals and subjects with cancer.

The M30 Apoptosense® ELISA and the M65® ELISA are valuable in researching subjects with cancer:

- ✔ The M30 Apoptosense® ELISA and the M65® ELISA can be used as tools to quantify levels of K18
- ✔ The M30 Apoptosense® ELISA can be of value when studying CK18 levels in subjects

EXAMPLE 1
Researchers studied pancreatic cancer and found that subjects with a high total K18 level, measured by the M65® ELISA (> 500 U/L), had a worse outcome than those with lower concentration. High K18 levels were associated with a poor prognosis.

EXAMPLE 2
Researchers studied K18 fragments and total K18 in gastric cancer. They showed that M30 Apoptosense® ELISA and the M65® ELISA could be used to stratify subjects into those with more or less progressive disease.

EXAMPLE 3
Researchers compared the K18 content of tumors (not serum) assayed with the M30 Apoptosense® ELISA with proliferation indices in subjects with breast cancer. Tumor growth was described as the balance between cell growth, quantified by an index reflecting cell proliferation, and apoptotic cell death, measured by the M30 Apoptosense® ELISA. They compared the ratio of proliferation index to apoptotic cell death (M30) with the disease stage, and found that a high proliferation index and a low tumor M30 level indicated a poorer prognosis.

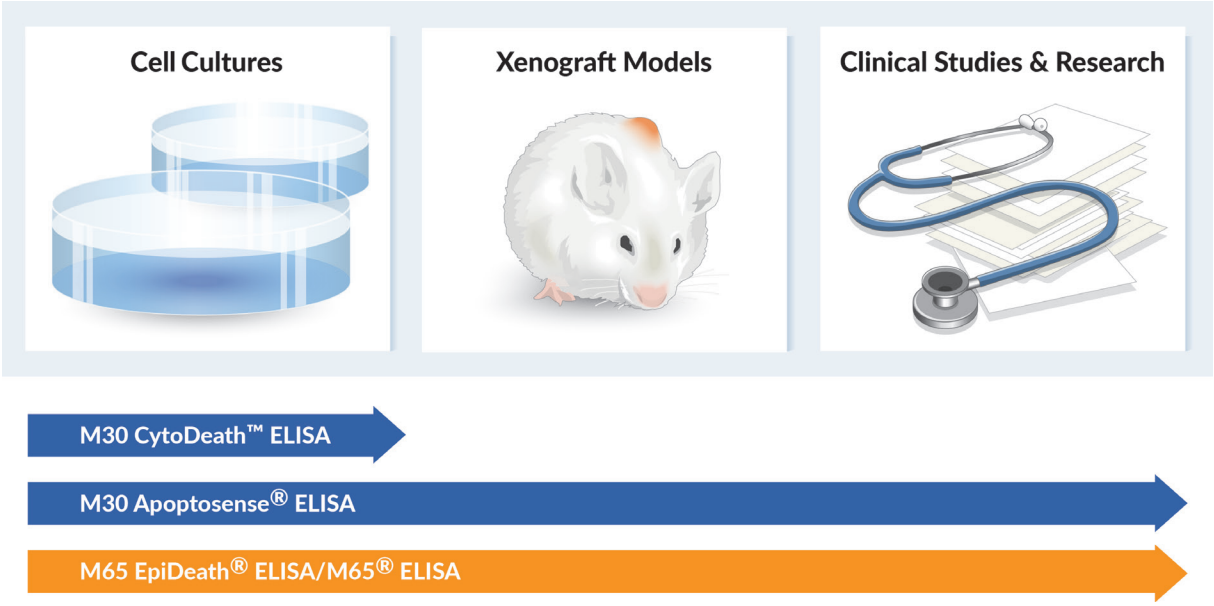
EXAMPLE 4
Researchers demonstrate that K18 fragment levels, measured with the M30 Apoptosense® ELISA, can discriminate between subjects with Hepatocellualr carcinoma and healthy individuals. Furthermore, M30 levels provide early information about the treatment response when treating subjects with Hepatocellular carcinoma.

Summary

The induction of apoptosis is an important mechanism in anti-cancer therapy. It causes the death of cancerous tissue while minimising the release of potentially toxic intracellular content via necrosis.

K18 fragments and total K18, measured by the M30® ELISAs and the M65® ELISA respectively, are sensitive and specific biomarkers for identifying and monitoring apoptosis and total cell death (apoptosis and necrosis). They are therefore valuable biomarkers in anti-cancer drug development.

The M30® ELISAs and the M65® ELISA can be used in the whole drug development process, making the PEVIVA products highly valuable in the Oncology Research field.



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The PEVIVA products

M30 Apoptosense® ELISA
(Prod. No P10011)

The M30 Apoptosense® ELISA measures the concentration of caspase-cleaved keratin 18 in human plasma, serum or cell culture supernatants, reflecting the amount of apoptosis. The assay is based on the unique M30 antibody, which recognizes a neo-epitope of keratin 18 formed after caspase cleavage. The assay can be combined with the M65® ELISA for the analysis of cell death mode (necrosis or apoptosis).

All reagents are provided in a convenient ready-to-use format.

M30 CytoDeath™ ELISA
(Prod. No P10900)

The M30 CytoDeath™ ELISA offers a unique possibility to measure apoptotic cells in multicellular spheroids and organ culture systems. The M30 CytoDeath™ ELISA is a product developed for cell culture applications, with a dynamic range and sensitivity suitable for in vitro work, making it a useful drug screening tool.

Similar to the M30 Apoptosense® ELISA, the M30 CytoDeath™ ELISA is based on the M30 antibody, detecting the caspase-cleaved keratin 18. All reagents are provided in a convenient ready-to-use format.

M65® ELISA
(Prod. No P10020)

The M65® ELISA measures soluble keratin 18 released from dying cells. It can be used to assess overall cell death, due to apoptosis or necrosis. The M65® ELISA is intended for human serum or plasma, and is CE marked as a medical device for in vitro diagnostic use.

The M65® ELISA is primarily intended to be used together with the M30 Apoptosense® ELISA. When used together, the quantification of total cell death, apoptosis and necrosis is possible. As both assays are calibrated against the identical reference, the combination of the M30 Apoptosense® ELISA and the M65® ELISA allows determination of the relative contribution of apoptosis to total cell death. All reagents are provided in a convenient ready-to-use format.

M65 EpiDeath® ELISA
(Prod. No P10040)

The M65 EpiDeath® ELISA measures the concentration of soluble keratin 18 in human plasma, serum and cell culture supernatants. The keratin 18 levels reflect the amount of total cell death, due to apoptosis or necrosis.

The M65 EpiDeath® ELISA represents the next generation of keratin 18 positive biomarkers. All reagents are provided in a convenient ready-to-use format.

How to order

VLVbio is collaborating with distributors all around the world to provide fast, reliable and convenient service. In the US and Canada, please visit Diapharma on the web at **diapharma.com** or e-mail **info@diapharma.com.** You can also e-mail VLVbio directly at **info@vlvbio.com.**

Other PEVIVA Line Products	
ELISA Products	Prod. No
M30 Apoptosense® ELISA	P10011
M30 CytoDeath™ ELISA	P10900
M65® ELISA	P10020
M65 EpiDeath® ELISA	P10040
Monoclonal Antibody Products	Prod. No
M5 Keratin 18 mAb	P10600
M6 Keratin 18 mAb	P10650
M30 CytoDEATH™ mAb (unlabelled)	P10700
M30 CytoDEATH™ mAb Biotin	P10750
M30 CytoDEATH™ mAb Fluorescein	P10800
M30 CytoDEATH™ mAb Orange	P10830

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