# **APOPTOSIS:**

# 🍃 PEVIVA

Apoptosis

### Assays for the accurate measurement of caspase-cleaved keratin 18 A biomarker of apoptosis

#### M30 Apoptosense<sup>®</sup> ELISA & M30 CytoDeath<sup>™</sup> ELISA

The M30 Apoptosense<sup>®</sup> ELISA and M30 CytoDeath<sup>™</sup> ELISA are perfect tools for measurement of apoptosis of human epithelial cells. The assays quantify the accumulation of soluble caspase-cleaved keratin 18 (ccK18), a product of apoptosis, in serum/plasma and cell culture supernatant or lysates, respectively. Keratin 18 (K18) is an intracellular protein, expressed by human epithelial cells, for example in the liver, lung, prostate, ovary, breast, GI-tract, and kidney. As a hallmark of apoptotic mechanisms in the cell, caspases are activated and cleave K18 into fragments (figure 1) at specific sites. The M30 antibody is specific for a neo-epitope exposed after caspase cleavage of K18 at Asp396 and is therefore a reliable marker for apoptosis. The M30 assays are in a convenient ready-to-use format and can be split up for use at several occasions.

3 5 0 caspase-cleaved keratin 18 Asp396-Neoepitope M30 M5 or M6 measurement of cleaved K18 only

#### M30 Apoptosense/M30 CytoDeath **ELISA** measure only apoptosis

Figure 1. The figure shows how intact keratin 18 (K18) is cleaved by caspases into fragments upon apoptosis in human epithelial cells. The product of this event can be used as a specific marker for apoptosis in cell culture supernatants, serum and plasma.

The M30 Apoptosense® ELISA is our most popular product because of its reliable and reproducible quantitative detection of apoptosis. It can be used together with the M65<sup>®</sup> ELISA for the quantification and differentiation of cell death mode by comparison of apoptosis and necrosis levels (figure 2). Both assays are calibrated against the same standard peptide, allowing them to determine the ratio between apoptosis and necrosis. This technique is ideal when investigating which cell death mode a certain disease, pharmaceutical or toxin has induced. The M30 Apoptosense® ELISA is CE marked as a medical device for in vitro diagnostic use within the EU (for research use only in the US).

The M30 CytoDeath<sup>™</sup> ELISA is a research product developed for cell culture applications and is suitable for *in vitro* work. M30 CytoDeath<sup>™</sup> ELISA is a powerful drug screening tool for *in vitro* characterisation of apoptosis-inducing drugs, including establishment of kinetics and dose-response relationships.



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Main applications fields are hepatology, toxicology and oncology.



M30 Apoptosense® ELISA and M65® ELISA, representing levels of

apoptosis and total cell death, respectively.



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# M30 Apoptosense® ELISA CE

Cat. No	10011
Test	96 determinations: 7 standards, 2 controls and 39 samples in duplicate
Method	ELISA
Range	0 – 1 000 U/L (units are defined against a synthetic peptide containing the M30 and M5 epitopes; 1 U/L = 1,24 pM). Working range: 75 – 1 000 U/L
Sensitivity	20 U/L
Incubation time	260 min (approx.)
Sample volume	25 μL (duplicates recommended)
Reagent storage	+2 - 8 °C. Do not freeze!
Sample type	Human serum or plasma (EDTA, citrate, heparin plasma), K18-reactive material released from K18 positive (epithelial cells) apoptotic human cells. Multiple freeze-thaw cycles of samples are well tolerated. The same type of material, i.e. serum or plasma collected by one method, should be used for a specific project. Cell culture supernatants from K18 positive cells or tissues.
Sample preparation	If the assay is to be performed the same day, the samples can be stored at $2 - 8$ °C.Samples are stable for at least 9 months at -20 °C, and for at least two years when stored at -80 °C.
Reference range	In serum from 200 Swedish blood donors, the median level was 94 U/L, range: 19 – 668 U/L. The 95 <sup>th</sup> percenti- le was 251 U/L. It is recommended that each laboratory establish their own reference range.
Species	Human
Specificity	The assay uses two monoclonal antibodies, the M30 antibody directed against the K18Asp396 neo-epitope and the M5 antibody directed against the 284 – 396 region of the K18 protein. Soluble caspase-cleaved K18 (ccK18) that exposes these epitopes will be detected by the assay.

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### M30 CytoDeath™ ELISA

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Cat. No	10900
Test	96 determinations: 4 standards and 44 samples in duplicate
Method	ELISA
Range	0 – 3 000 U/L (units are defined against a synthetic peptide containing the M30 and M6 epitopes; 1 U/L = 1,24 pM). Working range: 250 – 3 000 U/L
Sensitivity	60 U/L
Incubation time	260 min (approx.)
Sample volume	25 μL (duplicates recommended)
Reagent store	+2 - 8 °C. Do not freeze!
Sample type	The M30 CytoDeath <sup>™</sup> ELISA detects apoptosis in <i>in vitro</i> cell cultures. K18-reactive material released from K18 positive (epithelial cells) human cells.
Sample preparation	If the assay is to be performed the same day, the samples can be stored at 2 – 8 °C. Samples are stable for at least 9 months at -20 °C, and for at least two years when stored at -80 °C.
Species	Human
Specificity	The assay uses two monoclonal antibodies, the M30 antibody directed against the K18Asp396 neo-epitope and the M5 antibody directed against the 284 – 396 region of the K18 protein. Soluble caspase-cleaved K18 (ccK18) that exposes these epitopes will be detected by the assay.