

M30 Apoptosense® ELISA and M65 EpiDeath® ELISA

# ***SERUM BIOMARKERS*** **for** ***TOXIC LIVER INJURY***

## **Determination of hepatocyte cell death by the M30 Apoptosense® ELISA and M65 EpiDeath® ELISA**

Adverse drug reactions (ADR) are of major concern in drug development. A common form of ADR is liver toxicity, caused by the metabolism of drugs in hepatocytes.

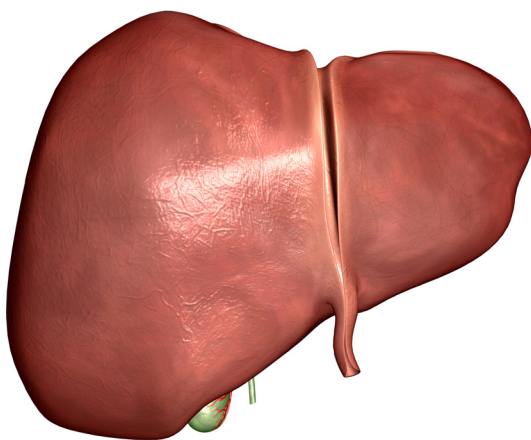
Drugs (or their metabolites) may trigger apoptosis or necrosis of hepatocytes. Different toxic stimuli are expected to be associated with different cell death modes.

Keratin 18 (K18) biomarkers can be used to determine cell death modes in plasma

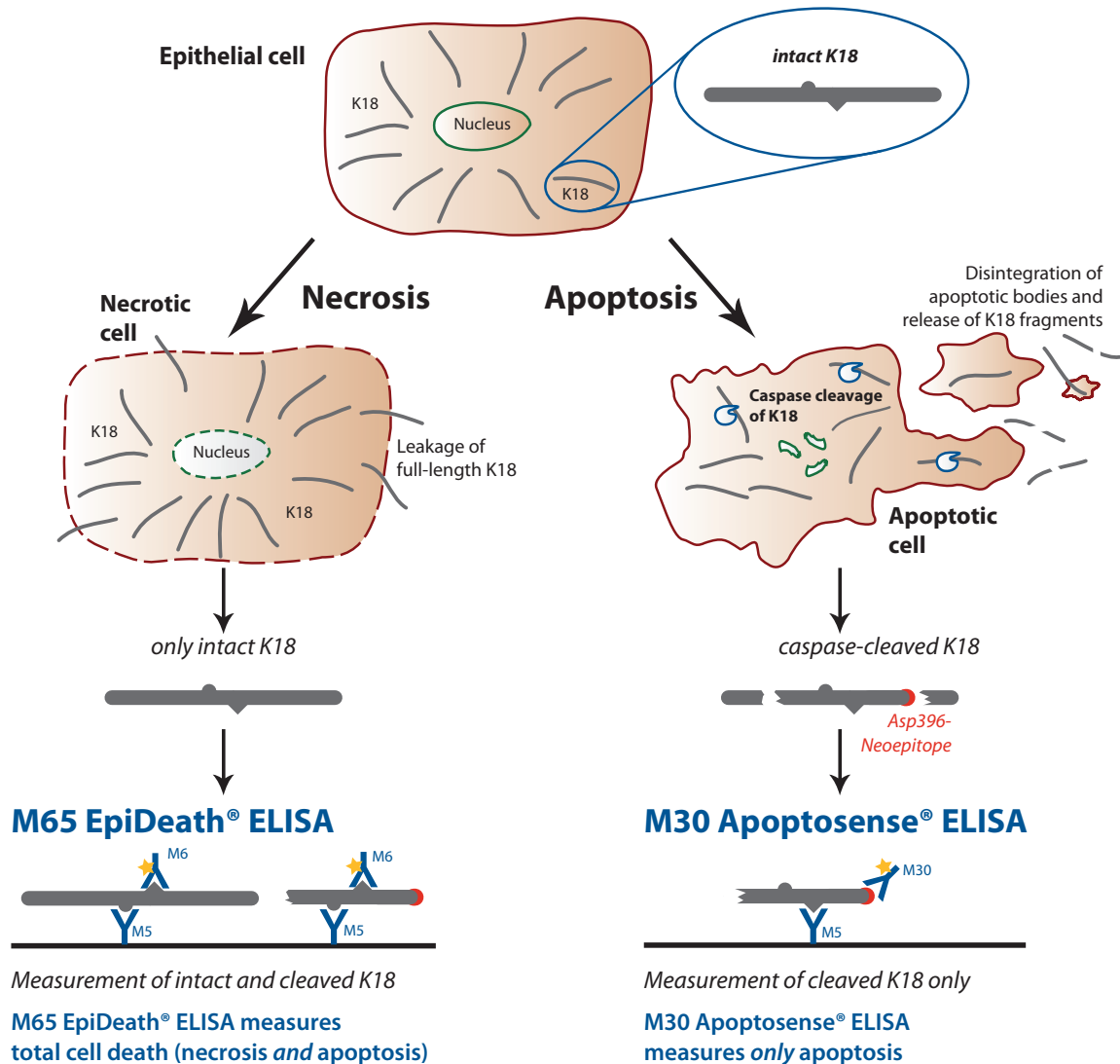
samples. Caspase-cleaved K18 reflects apoptotic cell death, whereas total K18 is a measure of total cell death (necrosis and apoptosis). Apoptosis can be measured using the M30 Apoptosense® ELISA and the M65 EpiDeath® ELISA quantifies total cell death.

The M30 Apoptosense® ELISA has been extensively used to show that serum/plasma levels of caspase-cleaved K18 are elevated in patients with liver injuries. A major advantage of this approach is that the analyte measured by the M30 Apoptosense® ELISA is an end product of the apoptotic process reflecting the extent of active disease (ongoing apoptosis).

K18 is a cytoskeletal protein which is abundantly expressed in hepatocytes. The liver is a large organ and hepatocytes are in direct contact with blood. Therefore, plasma K18 is likely to be derived from liver tissue in patients with no other diseases (such as large tumours or renal failure).



# Keratin 18 in necrotic and apoptotic cell death



**Apoptosis** is a regulated form of cell death. Intracellular proteins including keratin 18 are degraded by a class of proteases called caspases. K18 is cleaved at amino acid Asp396.

**Necrosis** is an uncontrolled form of cell death characterized by disintegration of the membrane and leakage of cell content into the extracellular compartment.

During late phases of apoptosis, intracellular protein fragments may be released into the extracellular compartment and into the blood stream.

Both the cleaved and uncleaved forms of K18 are relatively stable in blood. The levels of total K18 reflect total cell death due to either necrosis or apoptosis, whereas the levels of caspase-cleaved K18 reflect apoptosis.

The different molecular forms of K18 can be determined by specific ELISA assays. The M30 Apoptosense® ELISA utilizes the M30 monoclonal antibody, which is specific for the Asp396 neo-epitope and does not bind to the native protein. Therefore, the M30 Apoptosense ELISA is specific for apoptosis.

The M65 EpiDeath® ELISA measures total K18. The assay uses two monoclonal antibodies directed against conventional epitopes on K18 and measures both cleaved and uncleaved K18.

The combination of the M30 Apoptosense® ELISA and the M65 EpiDeath® ELISA allows both quantitative and qualitative determination of cell death.

## APAP hepatotoxicity correlates with K18 levels

Antoine *et al.*, 2009 studied the use of K18 as a serum biomarker for drug induced liver injury. The concentrations of caspase-cleaved and native K18 were significantly increased after induction of acetaminophen (APAP) induced liver toxicity. The values correlated strongly with histological changes and *in situ* cell loss and the time-course of injury.

Interestingly, the ratio between caspase-cleaved and full-length K18 indicated a change of cell death mode over time from an apoptotic onset to cell death dominated by necrosis in later stages.

Increases of K18 serum levels correlated well with ALT levels, but were observed at earlier timepoints. Generally, K18 was reported to be a sensitive and specific indicator of hepatotoxicity.

### Reference

Antoine *et al.*, High-mobility group box-1 protein and keratin-18, circulating serum proteins informative of acetaminophen-induced necrosis and apoptosis *in vivo*. *Toxicol Sci.* 2009 112: 521 – 31.

## Toxicant induced liver injury

Vinyl chloride (VC) is a potent liver toxic chemical widely used in the industry. VC workers exposed to VC on a regular basis may develop liver steatosis and fibrosis.

Liver injuries in VC workers, verified by liver biopsies, were reported to correlate with K18 blood concentrations, despite normal ALT values (Cave *et al.*, 2010). An influence of obesity or alcohol abuse could be ruled out, leaving the toxic effect of VC as the only reasonable cause for the liver diseases.

Both forms of K18 were analyzed and necrosis was found to be the mechanism of cell death. Monitoring serum K18 levels should be useful for identification of individuals that are being exposed to harmful amounts of toxic chemicals.

### Reference

Cave *et al.*, Toxicant-associated steatohepatitis in vinyl chloride workers. *Hepatology.* 2010 51: 474–81.



## K18 levels are elevated in ALF and NASH

Apoptosis is the major mechanism leading to fibrosis in liver diseases such as alcoholic (ASH) or non-alcoholic (NASH) steatohepatitis and hepatitis B or C virus infection. As a result, caspase-cleaved K18 concentrations are significantly elevated in patients with these diseases.

In blood samples from patients with acute liver failure (ALF), the concentrations of both forms of K18

are highly elevated. It has been reported that the distinction between apoptosis and necrosis may be useful for predicting the outcome of ALF.

### References

Volkman *et al.*, Caspase activation is associated with spontaneous recovery from acute liver failure. *Hepatology.* 2008 47: 1624–33.

## M30 Apoptosense® ELISA

- Specific measurement of apoptosis in epithelial cells (hepatocytes)
- Samples: Human serum or plasma, or cell culture supernatants or cell lysates from human cell lines; no sample preparation required
- Sample stability: at least 1 day at 2 – 8 °C, at least 9 months at -20 °C, at least 2 years at -80 °C; freeze-thawing is well tolerated
- Sandwich ELISA with precoated microplate, 12 × 8 wells; kit includes all necessary reagents
- Registered in accordance with the CE directive; manufactured according to ISO 9001 and ISO 13485 guidelines



## M65 EpiDeath® ELISA

- Specific measurement of total cell death in epithelial cells (hepatocytes)
- Samples: Human serum or plasma, or cell culture supernatants from human cell lines; no sample preparation required
- Sample stability: at least 1 day at 2 – 8 °C, at least 9 months at -20 °C, at least 2 years at -80 °C; freeze-thawing is well tolerated
- Sandwich ELISA with precoated microplate, 12 × 8 wells; kit includes all necessary reagents
- Registered in accordance with the CE directive; manufactured according to ISO 9001 and ISO 13485 guidelines



### Products from PEVIVA

#### M30 Apoptosense® ELISA

Prod. no. 10010

#### M65® ELISA

Prod. no. 10020

#### M65 EpiDeath® ELISA

Prod. no. 10040

#### M30 CytoDeath™ ELISA

Prod. no. 10900

#### M5 Keratin™ 18 mAb

Prod. no. 10600

#### M6 Keratin™ 18 mAb

Prod. no. 10650

#### M30 CytoDEATH™ mAb

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Fluorescein: Prod. no. 10800

Orange: Prod. no. 10830

Red: Prod. no. 10850

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