DiaPharma Group, Inc.

We specialize in biomarkers for toxicology (drug-induced liver and kidney injury), liver disease, oncology, and hemostasis

Featured Products are K18 biomarkers from VLVBio AB

VLVbio AB combines the innovative will and energy of a company founded in 2013 with the history and built-in expertise of a Product Line invented more than a decade ago at the Karolinska Institute in Stockholm, Sweden.
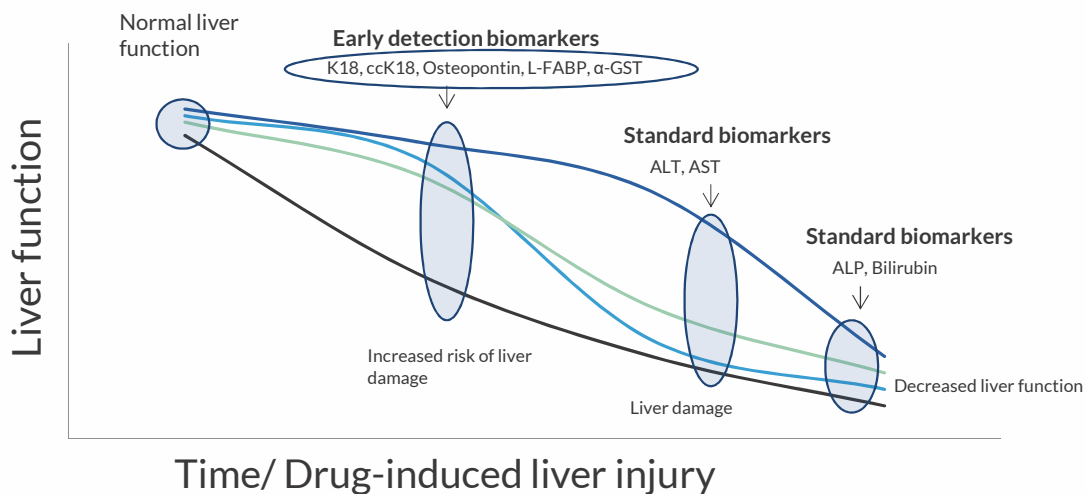
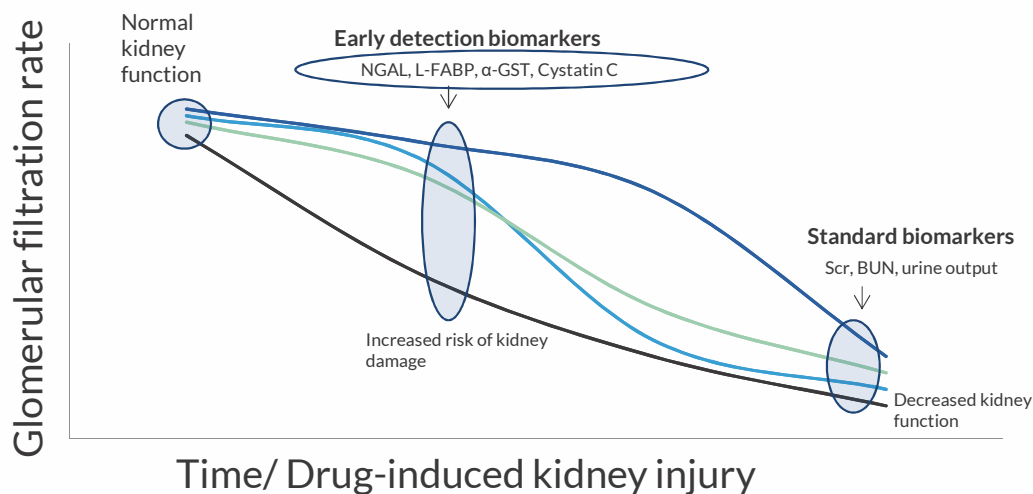Bringing innovative high-quality biomarker assays to DILI research.

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Biomarkers of Drug-Induced Liver Injury

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Biomarkers of Drug-Induced Kidney Injury

Glomerular filtration rate (y-axis) vs. Time/ Drug-induced kidney injury (x-axis)

Normal kidney function

Early detection biomarkers
NGAL, L-FABP, α-GST, Cystatin C

Standard biomarkers
Scr, BUN, urine output

Increased risk of kidney damage

Decreased kidney function

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Use of new biomarkers

Featuring Keratin 18

- Increase tissue specificity

- Provide mechanistic information

- Predict liver injury at earlier time points than ALT

- Current uses:
  - K18 as a secondary level of investigation if the first biomarkers indicate liver issues
    - Company has already identified a potential liver signal and is trying to gain some mechanistic understanding about the signal. (eg, observed elevated ALT to determine if the injury is primarily necrotic or apoptotic)
  - K18 as a first line in cases where liver toxicity is a known issue
    - (ie, immuno-oncology drug investigations)

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Keratin 18 in the human body

Almost all cells of epithelial origin:

- **Liver**
- Lung
- Intestines
- Kidney
- Breast tissue
- Prostate
- Ovary

Breast tissue
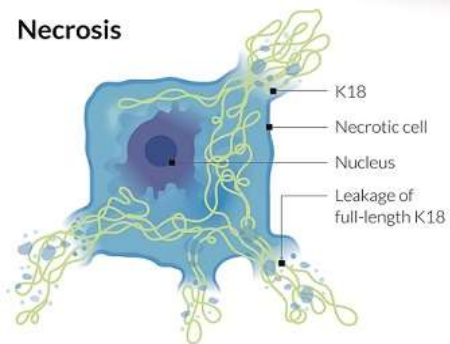
Lung

Liver

Kidney

Intestines

Ovary

Prostate
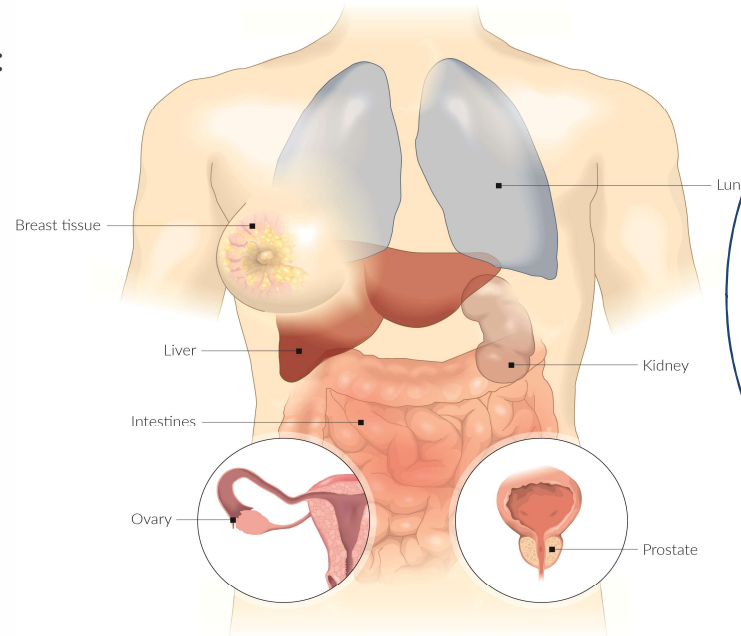
- Brain/CNS/ Neurons
- Skin
- Muscles
- Connective tissue
- Bone marrow
- Blood

PEVIVA

Apoptosis and Necrosis – K18

Hepatocyte cell death

- K18
- Nucleus
- Hepatocyte cell
- Intact K18

Necrosis
- K18
- Necrotic cell
- Nucleus
- Leakage of full-length K18

Apoptosis
- Apoptotic cell
- Caspase cleavage of K18
- Caspase
- Disintegration of apoptotic bodies and release of K18 fragments

Only intact K18

Caspase-cleaved K18

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Apoptosis and Necrosis

Only intact K18	Caspase-cleaved K18
	Asp396-Neoepitope
M65® ELISA **M65 EpiDeath® ELISA**	**M30 Apoptosense® ELISA** **M30 CytoDeath™ ELISA**
M5 M5 M6 M6	M5 M30
Measurement of intact and cleaved K18	Measurement of cleaved K18 only
The M65 ELISAs measure total cell death (necrosis and apoptosis)	The M30 ELISAs measure only apoptosis

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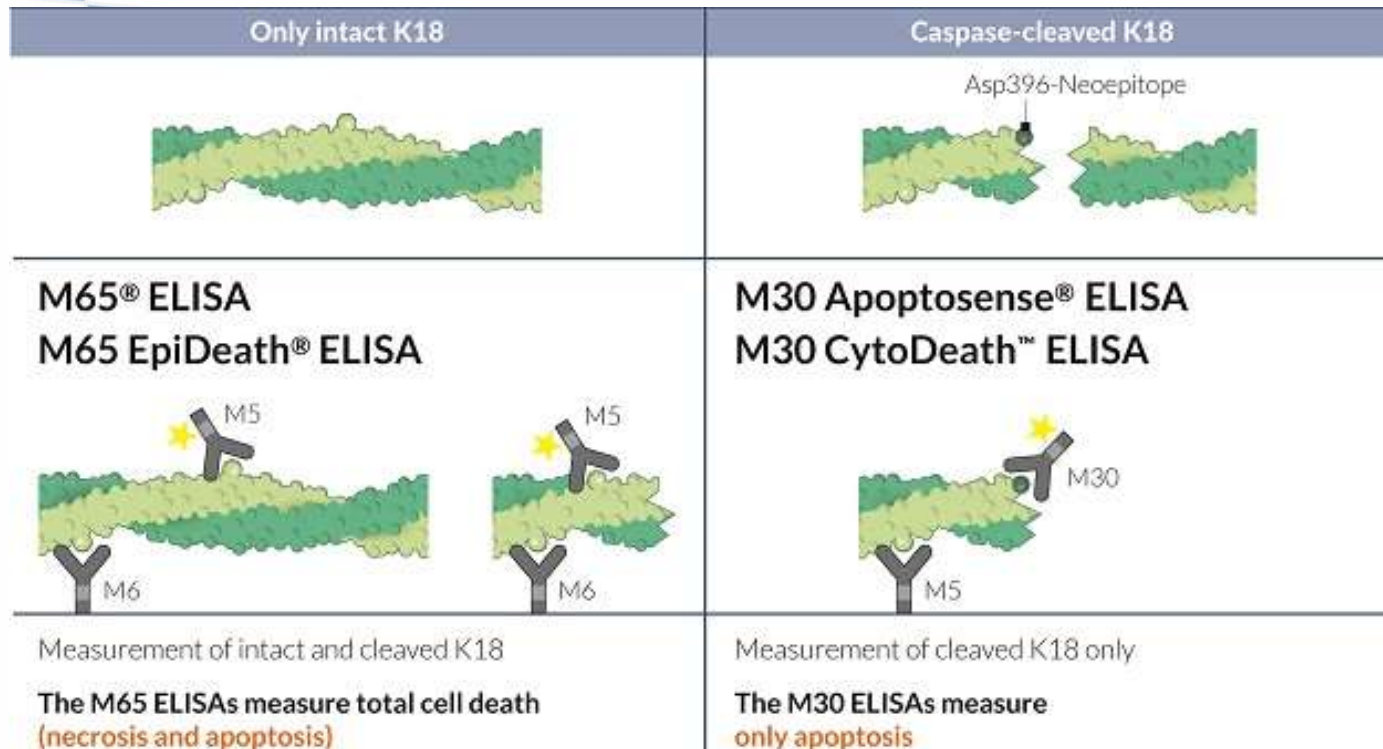
PEVIVA Product Line

Overview

ELISA Products
- M30 Apoptosense® ELISA
- M30 CytoDeath™ ELISA
- M65® ELISA
- M65 EpiDeath® ELISA

Antibody Products
- M30 CytoDEATH ™ mAb
  M6 & M5 Keratin 18 mAb

**VLV**bio

PEVIVA Product Line

Overview

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

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PEVIVA Product Line

Determination of Cell Death Mode

- Products can be useful to quantify:
  - Apoptosis alone (M30)
  - Total cell death (M65)

  - Apoptosis and necrosis (M30 and M65)

Apoptosis

M30 level   ccK18

M65 level   total K18
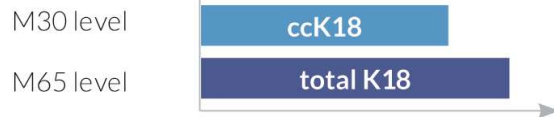
High levels of caspase-cleaved K18 (ccK18)
compared to total K18 (high M30:M65 ratio)

Necrosis

M30 level   ccK18

M65 level   total K18

Low levels of caspase-cleaved K18 (ccK18)
compared to total K18 (high M30:M65 ratio)

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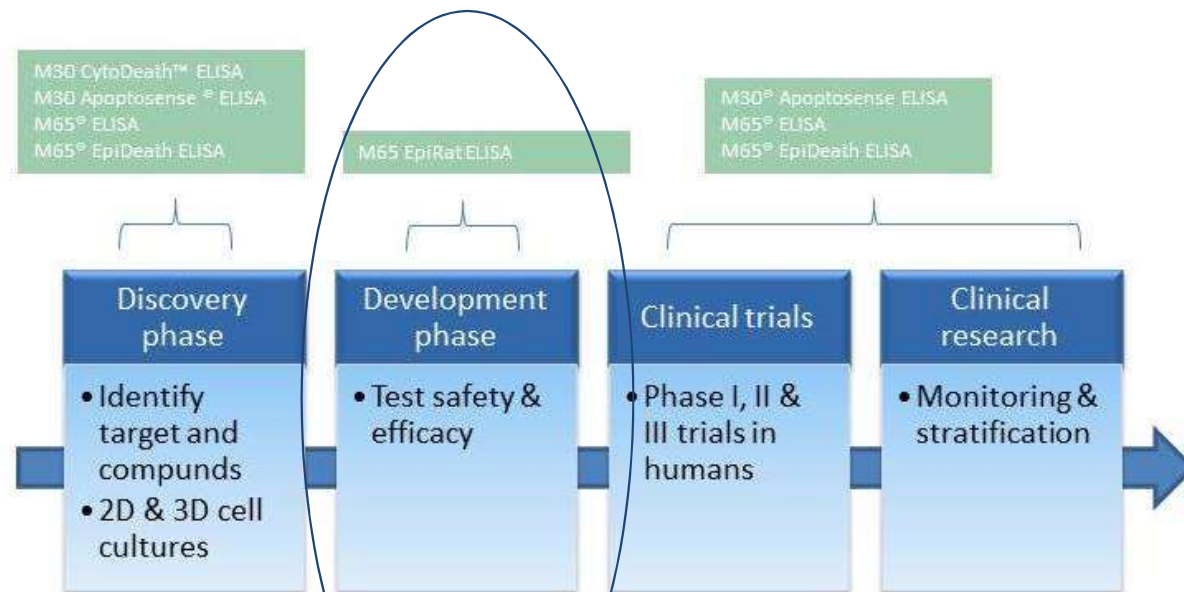
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Drug-Induced Liver Injury Biomarker

Numerous ongoing research for use of K18 in DILI

- **Letters of Support – EMA and FDA: Keratin 18 and Osteopontin**
- **Comparison to traditional biomarkers (ie, ALT) with APAP or exercise**
- **Drugs (ie, Heparins) showing elevations in ALT/AST, miR-122 without presence of clinically significant liver injury**
- **Drugs meeting stopping criteria as outlined by FDA for potential hepatotoxicity may not results in serious liver injury with use of new biomarkers and mathematical modeling (ie, Cimaglermin alfa)**
- **Toxicant-Associated Steatohepatitis (TASH)**
- **Alcohol related liver damage**

→ All of the above research studies using human samples. Previous K18 biomarkers did not detect mouse or rat K18 resulting in limitations in various preclinical models

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Introduction of M65® EpiRat ELISA

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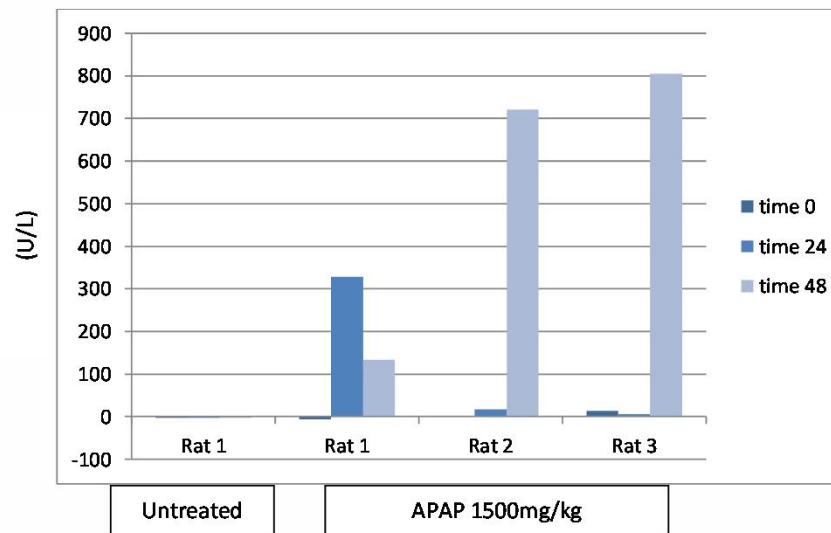*The dead cells still count!* ™

M65® EpiRat ELISA

- Production of the new rodent-detecting antibody (monoclonal).

- 95 clones selected after mice immunization, 7 clones selected for further testing.

- Only rat, *does not detect K18 from mouse*!

- M65 EpiRat is born!

- The M65 EpiRat™ ELISA is based on the <u>M5 and M6 antibodies</u>, which bind to the biomarker Keratin-18 (K18). The concentration of K18 reflects the <u>amount of overall cell death, both apoptosis and necrosis</u>, in K18 positive cells. Levels of K18 are commonly elevated when the liver endures damage, through disease or toxicological effects.

For research and laboratory use only.
Not for human or diagnostic use.

M65 EpiRat ™ **ELISA**

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M65 EpiRat™ ELISA treated with Acetaminophen

Serum samples from untreated rats and rats treated with different doses of APAP were run using the M65 EpiRat™ ELISA. Below you can see results from two runs where rats treated with 1000mg/kg APAP and 1500mg/kg APAP compared to untreated controls. Blood was drawn after 0, 24, and 48 hours.

ELISAs for Drug-Induced Liver Injury (DILI)

ELISAs for Drug-Induced Liver Injury (DILI)

Biomarker	Sample Type	Description	Available Assays	Product #
Keratin 18 (K18)	Serum or plasma	Highly specific biomarker for hepatocyte cell death (necrosis and apoptosis)	Peviva M65® ELISA Peviva M65® Epideath® ELISA	P10020 P10040
Caspase-cleaved Keratin 18 (ccK18)	Serum or plasma	Highly specific biomarker for hepatocyte apoptosis	Peviva M30® Apoptosense	P10011
Osteopontin (OPN)	Serum or plasma	Biomarker for hepatic inflammation and necrosis	Osteopontin Osteopontin N-Half	DPG27158 DPG27258
Alpha Glutathione S-Transferase (α-GST)	Serum or plasma	Short half-life; sensitive to rapid changes in liver damage	TECO Alpha® GST	TE1056
Liver Fatty Acid Binding Protein (L-FABP, FABP1)	Serum or plasma	Sensitive marker for hepatic injury (leakage)	Diapharma L-FABP	DPGLFABP

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ELISAs for Drug-Induced Kidney Injury (DIKI)

ELISAs for Drug-Induced Kidney Injury (DIKI)

Biomarker	Sample Type	Description	Available Assays	Product #
Alpha Glutathione S-Transferase (α-GST)	Urine	Biomarker for proximal tubule damage	TECO Alpha® GST	TE1056
Osteopontin (OPN)	Urine	Biomarker for damage to loop of Henle and distal tubule	Osteopontin Osteopontin N-Half	DPG27158 DPG27258
Liver Fatty Acid Binding Protein (L-FABP, FABP1)	Urine	Marker of acute kidney injury; useful for early detection of renal damage accompanying tubular dysfunction	Urinary L-FABP (FABP1)	006
Cystatin C (Cys C)	Serum, plasma, or urine	Assay for estimation of the glomerular filtration rate (GFR); more sensitive than serum creatinine tests	IMD Rapid Cystatin C	31241
Neutrophil Gelatinese-Associated Lipocalin (NGAL)	Urine	Biomarker for distal tubular damage	Diapharma NGAL (96) Diapharma NGAL (2 x 96) IMD Lipocalin-2	DPG-NGAL DPG-2NGAL 31050
Alpha Klotho (α-Klotho)	Plasma	Biomarker of impaired renal function, cytoprotective	FluoBolt™ α-KLOTHO (MEF-FIA)	FIA-1704

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Questions?

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