

# M65 EpiDeath<sup>®</sup> ELISA

## Apoptosis + Necrosis Biomarker Assay

Catalog Prod. No. 10040

### General Information

In USA, Canada and Japan: For research and laboratory use only. Not for human or diagnostic use.

- Analyte:** Soluble human intermediate filament protein keratin 18 (K18) [Cytokeratin-18]. The assay uses two monoclonal antibodies directed to epitopes in the 284 - 396 region of the K18 protein. Soluble full-length as well as apoptotic fragments of keratin 18 (cck18) and protein complexes that expose these epitopes will be detected by the assay.
- Intended Use:** Quantitative measurement of total soluble cytokeratin 18 (K18) released from dead cells (necrotic and apoptotic). The cells or tissues should be of human epithelial origin (e.g. kidney, gut, colon, lung or liver) expressing K18.
- Samples:** **Human serum or plasma** (EDTA, Citrate, Heparin plasma), containing intact K18 or apoptotic K18 [K18Asp396-NE (M30)-reactive material] released from apoptotic K18 positive human cells. Multiple freeze-thaw cycles of samples are well tolerated.
- NOTE!** The same type of material i.e. serum or plasma collected by one method should be used for a specific project.
- Interfering Substances:** The assay is **not** sensitive to highly elevated hemoglobin levels (< 100 mg/dL), highly elevated triglyceride levels (< 1 250 mg/dL) or highly elevated bilirubin levels (< 12.5 mg/dL) allowing the analysis of even grossly haemolyzed, hyperlipidemic or icteric blood samples.
- Sample Volume:** 2 × 25 µL (duplicate samples).
- Sample Stability:** Fresh samples are stable for up to one day at 2–8 °C, for at least 9 months at -20 °C; and for at least two years when stored at -80 °C.
- Number of Tests:** 96 determinations: 8 Standards, 2 Controls and 38 samples in duplicates.
- Reagent Storage:** 2–8 °C. Do not freeze!
- Assay Time:** 270 min (approx.).
- References:**
- Younossi Z.M. et al.; (2008) *A novel diagnostic biomarker panel for obesity-related nonalcoholic steatohepatitis (NASH)*. *Obes Surg.* 18:1430-7
  - Hofer S. et al.; (2009) *Cell death serum biomarkers are early predictors for survival in severe septic patients with hepatic dysfunction*. *Crit Care.* 13:R93
  - Bechmann L.P. et al.; (2010) *Cytokeratin 18-based modification of the MELD score improves prediction of spontaneous survival after acute liver injury*. *J Hepatol.* 53:639-47
  - Tsutsui M. et al.; (2010) *Serum Fragmented Cytokeratin 18 Levels Reflect the Histologic Activity Score of Nonalcoholic Fatty Liver Disease More Accurately Than Serum Alanine Aminotransferase Levels*. *J Clin Gastroenterol.* 44:440-7
  - Lavallard V.J. et al.; (2011) *Serum markers of hepatocyte death and apoptosis are non-invasive biomarkers of severe fibrosis in patients with alcoholic liver disease*. *PLoS One.* 6:e17599
  - Joka E. et al.; (2012) *Prospective Biopsy-Controlled Evaluation of Cell Death Biomarkers for Prediction of Liver Fibrosis and Nonalcoholic Steatohepatitis*. *Hepatology.* 40:651-655
  - Brenner T. et al.; (2012) *Cell Death Biomarkers as Early Predictors for Hepatic Dysfunction in Patients After Orthotopic Liver Transplantation*. *Transplantation.* 94:185
  - Cao W. et al.; (2012) *A novel pathophysiological-based panel of biomarkers for the diagnosis of nonalcoholic steatohepatitis*. *J Physiol Pharmacol.* 63:347-53
  - Waidmann O. et al.; (2013) *Diagnostic and prognostic significance of cell death and macrophage activation markers in patients with hepatocellular carcinoma*. *J Hepatol.* 59:769-79
  - Thulin P. et al.; (2013) *Keratin-18 and microRNA-122 complement alanine aminotransferase as novel safety biomarkers for drug-induced liver injury in two human cohorts*. *Liver Int.* [Epub ahead of print]

## Performance Characteristics

<b>Calibration:</b>	The units measured by the M65 EpiDeath® ELISA are defined against native antigen spiked into serum. Native antigen is calibrated against a recombinant protein standard. 1 U/L = 1.24 pM.
<b>Working Range:</b>	67 – 5 000 U/L
<b>Detection Limit:</b>	11 U/L, Standard A (0 U/L) + 2 S.D.
<b>Reference Range:</b>	Recommended reference values for application in chronic liver diseases (CLD): <150 U/L: healthy 150-200 U/L: slightly elevated > 200 U/L: elevated
<b>Reproducibility:</b>	Intra-Assay (WA) Precision: CV < 10 % for values > 300 U/L. Inter-Assay (BA) Precision: CV < 10 % for values > 300 U/L.
<b>Hook Effect:</b>	No high dose “hook effect” occurs before 200 000 U/L which is well above concentrations of K18 reactive material observed in human blood samples.

## Reagents

<b>Coated Microstrips:</b>	One Microplate, 96 dry wells (12 strips × 8 wells). The wells are coated with mouse monoclonal K18 antibody M5.
<b>HRP Conjugate:</b>	Concentrate. One vial containing mouse monoclonal M6 antibody (anti-K18) conjugated to horse-radish peroxidase (HRP).
<b>Conjugate Dilution Buffer:</b>	One vial containing phosphate buffer with protein stabilizers.
<b>Standards A – G:</b>	The values of the Standards A – G are 0, 200, 400, 800, 1 200, 2 000, 3 000 and 5 000 U/L, respectively.
<b>Control Low and High:</b>	Two vials containing K18-reactive recombinant standard material.
<b>TMB Substrate:</b>	One vial containing TMB (3,3',5,5'-Tetramethylbenzidine) Solution.
<b>Stop Solution:</b>	One vial containing 1.0 M sulfuric acid.
<b>Wash Tablet:</b>	One tablet for preparation of Wash Solution.

## PEVIVA Products from VLVbio

<b>M30 Apoptosense® ELISA</b> Prod. no. 10011	<b>M65® ELISA</b> Prod. no. 10020	<b>M5 Keratin 18</b> Prod. no. 10600	<b>M30 CytoDEATH™</b> Unconjugated Prod. No. 10700 Biotin Prod. No. 10750 Fluorescein Prod. No. 10800 Orange Prod. No. 10850
<b>M30 CytoDeath™ ELISA</b> Prod. no. 10900	<b>M65 EpiDeath® ELISA</b> Prod. no. 10040	<b>M6 Keratin 18</b> Prod. no. 10650	

For further information, please visit [www.peviva.com](http://www.peviva.com) - order online at [www.shop.peviva.com](http://www.shop.peviva.com)

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