

M65[®] ELISA

Apoptosis + Necrosis Biomarker Assay

Catalog Prod. No. 10020

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

General Information

- 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 epitops 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.
- The M65[®] ELISA may be combined with the M30 Apoptosense[®] ELISA (PEVIVA Prod. No. 10010) for the determination of cell death mode (apoptosis versus necrosis).
- 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 four hours 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: 7 Standards, 2 Controls and 39 samples in duplicates.
- Reagent Storage:** 2–8 °C. Do not freeze!
- Assay Time:** 140 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

Calibration:	The units measured by the M65 [®] ELISA are defined against a synthetic peptide containing the M5 and M6 monoclonal antibody epitopes. 1 U/L = 1.24 pM.
Working Range:	125 – 2 000 U/L
Detection Limit:	11 U/L, Standard A (0 U/L) + 2 S.D.
Reference Range:	In serum from 222 Swedish blood donors, the median level was 264 U/L with a range between 136–480 U/L. The 95 th percentile was 413 U/L. Recommended reference values for application in chronic liver diseases (CLD): > 450 U/L elevated
Reproducibility:	Intra-Assay (WA) Precision: CV < 10 % for values > 125 U/L. Inter-Assay (BA) Precision: CV < 10 % for values > 125 U/L.
Hook Effect:	No high dose “hook effect” occurs before 50 000 U/L which is well above concentrations of K18-reactive material observed in human blood samples.

Reagents

Coated Microstrips:	One Microplate, 96 dry wells (12 strips × 8 wells). The wells are coated with mouse monoclonal K18 antibody M6.
HRP Conjugate:	Concentrate. One vial containing mouse monoclonal M5 antibody (anti-K18) conjugated to horseradish peroxidase (HRP).
Conjugate Dilution Buffer:	One vial containing phosphate buffer with protein stabilizers.
Standards A – G:	The values of the Standards A – G are 0, 125, 250, 500, 750, 1 200 and 2 000 U/L, respectively.
Control Low and High:	Two vials containing K18-reactive recombinant standard material.
TMB Substrate:	One vial containing TMB (3,3',5,5'-Tetramethylbenzidine) Solution.
Stop Solution:	One vial containing 1.0 M sulfuric acid.
Wash Tablet:	One vial containing 10x concentrated Wash Solution.

PEVIVA Products from VLVbio

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

For further information, please visit www.peviva.com - order online at www.shop.peviva.com

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