

# M65<sup>®</sup> 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

<b>Analyte:</b>	Human intermediate filament protein keratin 18 (K18). The assay uses two monoclonal antibodies directed to epitopes in the 284–396 region of the K18 protein. Soluble full length K18 as well as K18 fragments and protein complexes that expose these epitopes will be detected by the assay.
<b>Intended Use:</b>	<p>Quantitative measurement of total soluble keratin 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.</p> <p>The M65<sup>®</sup> ELISA can be combined with the M30 Apoptosense<sup>®</sup> ELISA (PEVIVA Prod. No. 10010) for determination of cell death mode (apoptosis versus necrosis). Death mode can be determined in vitro and in serum from cancer patients (Kramer <i>et al.</i>, Cancer Res. 2004).</p>
<b>Samples:</b>	<p><b>Human serum or plasma</b> (EDTA, Citrate, Heparin plasma), containing K18-reactive material released from K18 positive human cells. Multiple freeze-thaw cycles of samples are well tolerated. <b>NOTE!</b> The same type of material i.e. serum or plasma collected by one method should be used for a specific project.</p> <p><b>Cell culture supernatants</b> from K18 positive (epithelial) cells or tissues.</p> <p><b>Mouse plasma</b> can be used for measurement of human xenografts. Please contact Peviva or see reference Olofsson <i>et al.</i>, Cancer Biomark., 2009 for further information.</p>
<b>Interfering Substances:</b>	The assay is <b>not</b> 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.
<b>Sample Volume:</b>	2 × 25 µl (duplicate samples).
<b>Sample Stability:</b>	Fresh samples are stable for up to 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.
<b>Number of Tests:</b>	96 determinations: 7 Standards, 2 Controls and 39 samples in duplicates.
<b>Reagent Storage:</b>	2–8 °C. Do not freeze!
<b>Assay Time:</b>	140 min (approx.).
<b>References:</b>	<ul style="list-style-type: none"> <li>■ Kramer G, <i>et al.</i>, (2004) Differentiation between Cell Death Modes using Measurements of Different Soluble Forms of Extracellular Cytokeratin 18. <i>Cancer Research</i> 64: 1751–1756.</li> <li>■ Linder S, <i>et al.</i>, (2004) Determining tumor apoptosis and necrosis in patient serum using cytokeratin 18 as a biomarker. <i>Cancer Lett.</i> 214, 1–9.</li> <li>■ Kramer G, <i>et al.</i>, (2006) Docetaxel induces apoptosis in hormone refractory prostate carcinomas during multiple treatment cycles. <i>Br J Cancer</i> 94: 1592–8.</li> <li>■ Cummings J, <i>et al.</i>, (2007) Qualification of M30 and M65 ELISAs as surrogate biomarkers of cell death: long term antigen stability in cancer patient plasma. <i>Cancer Chemother Pharmacol</i> 60(6):921–4.</li> <li>■ Olofsson M, <i>et al.</i>, (2007) Cytokeratin-18 is a useful serum biomarker for early determination of response of breast carcinomas to chemotherapy. <i>Clin Cancer Res.</i> 13: 3198–3206.</li> <li>■ Hou JM, <i>et al.</i> (2009) Tumorigenesis and neoplastic progression evaluation of circulating tumor cells and serological cell death biomarkers in small cell lung cancer patients undergoing chemotherapy. <i>Am J Pathol</i> 175:808–816.</li> <li>■ Bechmann, <i>et al.</i> (2010) Cytokeratin 18-based modification of the MELD score improves prediction of spontaneous survival after acute liver injury. <i>J Hepatol.</i> 53:639–47.</li> </ul>

## Performance Characteristics

<b>Calibration:</b>	The Units measured by the M65 <sup>®</sup> ELISA are defined against a synthetic peptide containing the M5 and M6 epitopes. 1 U/L = 1.24 pM.
<b>Working Range:</b>	125 – 2 000 U/L.
<b>Detection Limit:</b>	11 U/L, Standard A (0 U/L) + 2 S.D.
<b>Reference Range:</b>	In serum from 222 Swedish blood donors, the median level was 264 U/L with a range between 136 – 480 U/L. The 95 <sup>th</sup> percentile was 413 U/L. It is recommended that each laboratory establishes its own reference range.
<b>Reproducibility:</b>	Intra-Assay (WA) Precision: CV < 10 % for values > 125 U/L. Inter-Assay (BA) Precision: CV < 10 % for values > 125 U/L.
<b>Spike Recovery:</b>	The Standard provided with the kit contains recombinant material which is not comparable to the fragment measured in the sample and is not adequate for spiking recovery tests. The recovery for the recombinant K18 protein fragment observed in human serum/plasma will be higher than expected.
<b>Linearity/Dilution:</b>	Recovery for human sera when diluted 1:2 in M65 Standard A (0 Units/L): 126 % (average) and 116 – 139 % (range).  Recovery for human sera when diluted 1:1 to 1:10 in the M65 Sample Dilution Buffer: 106 % (average) and 100 – 117 % (range).  The M65 Sample Dilution Buffer is available directly from Peviva.
<b>Hook Effect:</b>	No high dose “hook effect” occurs before 50 000 U/L.

## Reagents

<b>Coated Microstrips:</b>	One Microplate, 96 dry wells (12 strips × 8 wells). The wells are coated with mouse monoclonal K18 anti-body M6.
<b>HRP Conjugate:</b>	Concentrate. One vial containing mouse monoclonal M5 antibody (anti-K18) conjugated to horseradish 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, 125, 250, 500, 750, 1 200 and 2 000 U/L, respectively.
<b>Control Low and High:</b>	Two vials containing reactive components in phosphate buffered FCS.
<b>TMB Substrate:</b>	One vial containing 22 mL of TMB (3,3',5,5'-Tetramethylbenzidine) Solution.
<b>Stop Solution:</b>	One vial containing 8 mL of 1.0 M sulfuric acid.
<b>Wash Solution:</b>	One vial containing 50 mL of concentrated Wash Solution.

## Products from Peviva

<b>M30 Apoptosense<sup>®</sup> ELISA</b> Prod. no. 10010	<b>M65<sup>®</sup> ELISA</b> Prod. no. 10020	<b>M5 Keratin 18</b> Prod. no. 10600	<b>M30 CytoDEATH<sup>™</sup></b> Unconjugated Prod. No. 10700
			Biotin Prod. No. 10750
<b>M30 CytoDeath<sup>™</sup> ELISA</b> Prod. no. 10900	<b>M65 EpiDeath<sup>®</sup> ELISA</b> Prod. no. 10040	<b>M6 Keratin 18</b> Prod. no. 10650	Fluorescein Prod. No. 10800
			Red Prod. No. 10830
			Orange Prod. No. 10850

For further, up-to-date information and to order, please visit [www.peviva.se](http://www.peviva.se).



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