

M30 Apoptosense[®] ELISA

Apoptosis Biomarker Assay

Catalog Prod. No. 10011

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

General Information

Analyte:	<p>Soluble human intermediate filament protein fragments of keratin 18 (K18) that contain the M30 neo-epitope (K18Asp396-NE).</p> <p>The M30 neo-epitope (K18Asp396-NE) is a sensitive and integrative indicator specific for epithelial cell death involving caspase-3, -7 or -9 activation.</p>
Intended Use:	<p>Quantitative measurement of the apoptotic cell death biomarker K18Asp396-NE ("M30 antigen" or "caspase cleaved keratin 18", ccK18) released from dying human epithelial cell lines <i>in vitro</i> or <i>in vivo</i>. The cells or tissues should be of human epithelial origin (e.g. kidney, gut, colon, lung or liver) expressing K18.</p>
Samples:	<p>Human serum or plasma (EDTA, Citrate, Heparin plasma), containing 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.</p> <p>Cell lysates or cell culture supernatants from K18 positive (epithelial) apoptotic cells or tissues.</p> <p>Mouse plasma can be used for measurement of human xenografts. Please contact VLVbio or see reference Olofsson <i>et al.</i>, Cancer Biomark., 2009 for further information.</p>
Interfering Substances:	<p>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.</p>
Sample Volume:	2 × 25 µL (duplicate samples).
Sample Stability:	Fresh samples are stable for up two days 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:	260 min (approx.).
References:	<ul style="list-style-type: none"> ■ Ueno T, <i>et al.</i> (2003) Measurements of an apoptosis product in sera of breast cancer patients. <i>Eur J Cancer</i> 39, 769–74 ■ Bantel H, <i>et al.</i>, (2004) Detection of apoptotic caspase activation in sera from patients with chronic HCV infection is associated with fibrotic liver injury. <i>Hepatology</i> 40: 1078–1087 ■ Cummings J, <i>et al.</i>, (2005) Validation of pharmacodynamic assays to evaluate the clinical efficacy of an antisense compound (AEG 35156) targeted to the X linked inhibitor of apoptosis protein XIAP. <i>Br J Cancer</i> 92: 532–8 ■ 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 ■ Luft <i>et al.</i>, (2007) Serum cytokeratin-18 fragments as quantitative markers of epithelial apoptosis in liver and intestinal graft-versus-host disease. <i>Blood</i> 110:4535–42 ■ Feldstein AE, <i>et al.</i>, (2009) Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: A multicenter validation study. <i>Hepatology</i> 50:1072–8 ■ Olofsson M, <i>et al.</i> (2009) Specific demonstration of drug-induced tumour cell apoptosis in human xenografts models using a plasma biomarker. <i>Cancer Biomark.</i> 5:117–25. ■ D'Arcy P, <i>et al.</i> (2011) Inhibition of proteasome deubiquitinating activity as a new cancer therapy. <i>Nat Med</i>, 17, 1636–40

Performance Characteristics

Calibration:	The units measured by the M30 Apoptosense® ELISA are defined against native antigen spiked into serum. The native antigen is calibrated against a recombinant protein standard containing the M30 and M5 epitopes. 1 U/L = 1.24 pM.
Working Range:	75 – 1 000 U/L
Detection Limit:	20 U/L, Standard A (0 U/L) + 2 S.D.
Reference Range:	In serum from 200 Swedish blood donors, the median level was 94 U/L with a range between 19 – 668 U/L. The 95 th percentile was 251 U/L. It is recommended that each laboratory establishes its own reference range.
Reproducibility:	Intra-Assay (WA) Precision: CV < 10 % for values > 100 U/L. Inter-Assay (BA) Precision: CV < 10 % for values > 100 U/L.
Spike Recovery:	Recovery of high standard when spiked into human blood samples: 109 % (average) and 78 – 125 % (range).
Linearity/Dilution:	Recovery for human sera containing K18Asp396-NE when diluted 1:1 to 1:10 in M30 Standard A (0 U/L): 107 % (average) and 82 – 129 % (range).
Hook Effect:	No high dose “hook effect” occur before 200 000 U/L which is well above concentrations of K18Asp396-NE (M30)-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 M5.
HRP Conjugate:	Concentrate. One vial containing 0.4 mL mouse monoclonal M30 antibody (anti-K18Asp396-NE) conjugated to horseradish peroxidase (HRP).
Conjugate Dilution Buffer:	One vial containing 11 mL of phosphate buffer with protein stabilizers.
Standards A – G:	The values of the Standards A – G are 0, 75, 150, 250, 500, 750 and 1 000 U/L, respectively.
Control Low and High:	Two vials containing M30-reactive recombinant standard material.
TMB Substrate:	One vial containing 22 mL of TMB (3,3',5,5'-Tetramethylbenzidine) Solution.
Stop Solution:	One vial containing 7 mL of 1.0 M sulfuric acid.
Wash Tablet:	One tablet for preparation of Wash Solution.

Peviva Products

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, up-to-date information and to order, please visit www.peviva.com.

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