

M30 Apoptosense[®] ELISA

Apoptosis Biomarker Assay

Catalog Prod. No. 10010

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

General Information

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| 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 Peviva 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: | <p>2 × 25 µL (duplicate samples).</p> |
| Sample Stability: | <p>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.</p> |
| Number of Tests: | <p>96 determinations: 7 Standards, 2 Controls and 39 samples in duplicates.</p> |
| Reagent Storage: | <p>2–8 °C. Do not freeze!</p> |
| Assay Time: | <p>260 min (approx.).</p> |
| 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: 532-538■ Volkmann X, <i>et al.</i>, (2006) Caspase activation is required for antiviral treatment response in chronic hepatitis C virus infection. <i>Hepatology</i> 43: 1311-1316■ 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. |

Performance Characteristics

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| Calibration: | The Units measured by the M30 Apoptosense® ELISA are defined against a synthetic peptide containing the M30 and M5 epitopes. 1 U/L = 1.24 pM. |
| Working Range: | 75 – 1 000 U/L |
| Detection Limit: | 25 U/L, Standard A (0 U/L) + 2 S.D. |
| Reference Range: | In serum from 236 Swedish blood donors, the median level was 132 U/L with a range between 68 – 342 U/L. The 95 th percentile was 260 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: | The Standard provided with the kit contains recombinant K18 with the K18Asp396-NE M30 epitope. This standard allows an accurate definition of Units for the M30 and M65 kits. However, recombinant K18 full-length protein or K18 protein fragments behave differently from the K18 protein fragments in blood samples and are therefore not considered adequate for spiking recovery tests. The recovery for the recombinant K18 protein fragment observed in human serum/plasma will be higher than expected. |
| Linearity/Dilution: | Recovery for human sera containing K18Asp396-NE when diluted 1:1 in M30 Standard A (0 U/L): 116 % (average) and 106 – 124 % (range). When diluted 1:10, the recovery was > 150 %. Recovery for human sera containing K18Asp396-NE when diluted 1:1 to 1:10 in blood donor serum: 97 % (average) and 81 – 106 % (range). Note: when > 1:1 dilutions of positive serum samples are required, blood donor serum should be used. |
| Hook Effect: | No high dose “hook effect” occur before 50 000 U/L which is well above concentrations of K18Asp396-NE (M30)-reactive material observed in human blood samples. |

Reagents

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| 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 12 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 8 mL of 1.0 M sulfuric acid. |
| Wash Solution: | One vial containing 50 mL of concentrated Wash Solution. |

Products from Peviva

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| M30 Apoptosense® ELISA Prod. no. 10010 | 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 Red Prod. No. 10830 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.se.



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