**M30 Apoptosense® ELISA**

**Apoptosis Biomarker Assay**

**Catalog Prod. No. 10011**

**General Information**

**Analyte:** Soluble human intermediate filament protein fragments of keratin 18 (K18) that contain the M30 neo-epitope (K18Asp396-NE).

The M30 neo-epitope (K18Asp396-NE) is a sensitive and integrative indicator specific for epithelial cell death involving caspase-3, -7 or -9 activation.

**Intended Use:** 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 *in vitro* or *in vivo*. 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 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.
- **Cell lysates or cell culture supernatants** from K18 positive (epithelial) apoptotic cells or tissues.
- **Mouse plasma** can be used for measurement of human xenografts. Please contact VLVbio or see reference Olofsson et al., Cancer Biomark., 2009 for further information.

**Interfering Substances:** The assay is **not** sensitive to highly elevated hemoglobin levels (< 100 mg/dL), highly elevated triglyceride levels (< 1250 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 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:**
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 95th 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
- **M30 CytoDeath™ ELISA**
  Prod. no. 10900
- **M65® ELISA**
  Prod. no. 10020
- **M65 EpiDeath® ELISA**
  Prod. no. 10040
- **M5 Keratin 18**
  Prod. no. 10600
- **M6 Keratin 18**
  Prod. no. 10650
- **M30 CytoDEATH™**
  Unconjugated Prod. No. 10700
  Biotin Prod. No. 10750
  Fluorescein Orange Prod. No. 10800
  Prod. No. 10850

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