

M30 Apoptosense® ELISA

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Apoptosis Biomarker Assay

Catalog Prod. No. 10011

General Information

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

Analyte: Soluble human intermediate filament protein fragments of keratin 18 (K18) [ccK18 (caspase-cleaved

Cytokeratin-18)] 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 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 M30 Apoptosense® ELISA may be combined with the M65® ELISA (PEVIVA Prod. No. 10020) for the

determination of cell death mode (apoptosis versus necrosis).

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.

Interfering Substances: The assay is <u>not</u> sensitive to highly elevated hemoglobin levels (< 100 mg/dL), highly elevated trigly-

ceride 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: $2 \times 25 \mu L$ (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: 270 min (approx.).

References:Linderholm BK, et al.; (2013) *Identification of intermediate risk breast cancer patients with 1-3 positive lymph nodes and excellent survival after tamoxifen as only systemic adjuvant therapy by use of mark-*

ers of proliferation and apoptosis. Breast. 22:643-9

■ De Petris L, et al.; (2011) Diagnostic and prognostic role of plasma levels of two forms of cytokeratin

18 in patients with non-small-cell lung cancer. Eur J Cancer. 47:131-7

■ Linder S, et al.; (2010) Utilization of cytokeratin-based biomarkers for pharmacodynamic studies.

Expert Rev Mol Diagn. 10:353-9 Review.

■ Brandt D, et al.; (2010) Serum biomarkers of cell death for monitoring therapy response of gastrointes-

tinal carcinomas. Eur J Cancer. 46:1464-73

Herrmann R, et al. (2008) Screening for compounds that induce apoptosis of cancer cells grown as

multicellular spheroids. J Biomol Screen. 13:1-8

■ Olofsson MH, et al.; (2007) Cytokeratin-18 is a useful serum biomarker for early determination of

response of breast carcinomas to chemotherapy. Clin Cancer Res. 13:3198-206

Kramer G, et al.; (2006) Docetaxel induces apoptosis in hormone refractory prostate carcinomas dur-

ing multiple treatment cycles. Br J Cancer. 94:1592-8

■ Bivén K, et al.; (2003) A novel assay for discovery and characterization of pro-apoptotic drugs and for

monitoring apoptosis in patient sera. Apoptosis. 8:263-8

Performance Characteristics

Calibration: The units measured by the M30 Apoptosense® ELISA are defined against a synthetic peptide containing

the M30 and M5 monoclonal antibody 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.

Reproducibility: Intra-Assay (WA) Precision: CV < 10 % for values > 100 U/L.

Inter-Assay (BA) Precision: CV < 10 % for values > 100 U/L.

Hook Effect: No high dose "hook effect" occurs before 50 000 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 anti-

K18 antibody M5.

HRP Conjugate: Concentrate. One vial containing mouse monoclonal M30 antibody (anti-K18Asp396-NE) 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, 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 TMB (3,3′,5,5′-Tetramethylbenzidine) Solution.

Stop Solution: One vial containing 1.0 M sulfuric acid.

Wash Tablet: One tablet for preparation of Wash Solution.

PEVIVA Products from **VLV**bio

M30 Apoptosense® ELISA M65® ELISA M5 Keratin 18 M30 CytoDEATH™

Prod. no. 10011 Prod. no. 10020 Prod. no. 10600 Unconjugated Prod. No. 10700
Biotin Prod. No. 10750
M30 CytoDeath™ ELISA M6 Keratin 18 Fluorescein Prod. No. 10800

M30 CytoDeath™ ELISAM65 EpiDeath® ELISAM6 Keratin 18FluoresceinProd. No. 10800Prod. no. 10900Prod. no. 10040Prod. no. 10650OrangeProd. No. 10850

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



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