M30 Apoptosense® ELISA
Apoptosis Biomarker Assay

Catalog Prod. No. 10011

General Information

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 the apoptotic cell death biomarker K18Asp396-NE ("M30 antigen" or caspase-cleaved (Cyto)keratin 18, ccK18) released from apoptotic epithelial cells / tissues (e.g. hepatocytes).

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 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.

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: 270 min (approx.).


Jain A. K. et al.; (2013) Serum keratin fragment 18 (CK18) levels significantly predict changes in liver histology in children and adolescents with nonalcoholic fatty liver disease (NAFLD): Results from the TONIC trial AASLD Presentation and Abstract #114 (2013)

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.

Reference Range: Recommended reference values for application in chronic liver diseases (CLD):
- <150 U/L: healthy
- 150-200 U/L: slightly elevated
- > 200 U/L: elevated

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.

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