PEVIVA

The dead cells still count!

M30 CytoDeath™ ELISA

Apoptosis detection in 2D and 3D cell cultures

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

Catalog Prod. No. 10900

General Information

Analyte: Soluble caspase-cleaved fragments of the intermediate filament protein keratin 18 (K18) containing the M30 neo-epitope (K18Asp396-NE). The assay detects human, monkey and bovine K18 fragments.

Intended Use: Quantitative measurement of the apoptotic cell death biomarker K18Asp396-NE in cell culture experiments. Can be used for cell lysates and/or culture supernatants. The assay only detects apoptosis in cells of epithelial origin that express K18. Cells should be of human, monkey or bovine origin.

To be used to determine accumulation of caspase-cleaved K18 in cell cultures, providing an integrative measure of apoptosis. The K18Asp396 neo-epitope is formed by caspase-3, -7 or -9 activation.

Samples: Cell lysates or culture supernatants. *Not suitable* for serum or plasma samples.

Sample Volume: 2 × 25 µl (duplicate samples).

Sample Stability: Fresh samples are stable for up to 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: 4 Standards and 44 samples in duplicates.

Reagent Storage: 2 – 8 °C. Do not freeze!

Assay Time: 260 min (approx.).

References:

- Fayad W, et al. (2009). *Identification of a novel topoisomerase inhibitor effective in cells overex- pressing drug efflux transporters*. PLoS One 4(10):e7238.
- Hernlund E, et al. (2009). *Ovarian carcinoma cells with low levels of b-F1-ATPase are sensitive to combined platinum and 2-deoxy-D-glucose treatment.* Mol Cancer Ther 8(7).
- Herrmann R, et al. (2008). *Screening for Compounds that Induce Apoptosis of Cancer Cells Grown as Multicellular Spheroids.* J Biomol Screen. 13(1):1 – 8.
- Lakshmikanthan V, et al. (2006). *SAHA-sensitized prostate cancer cells to TNFalpha-related apoptosis-inducing ligand (TRAIL): mechanisms leading to synergistic apoptosis.* Int J Cancer 119:221 – 8.
- Erdal H, et al. (2005). *Induction of lysosomal membrane permeabilization by compounds that activate p53-independent apoptosis.* Proc. Natl. Acad. Sci. USA 102, 192 – 197.
- Schutte B, et al. (2004). *Keratin 8/18 breakdown and reorganization during apoptosis*. Exp Cell Res. 297, 11 – 26.
- Kramer G, et al. (2004). *Differentiation between Cell Death Modes using Measurements of Different Soluble Forms of Extracellular Cytokeratin 18*. Cancer Research 64, 1751 – 1756.
- Hägg, M. et al. (2002). *A novel high-through-put assay for screening of pro-apoptotic drugs.* Invest. New Drugs, 20: 253-259.
- Leers MP, et al. (1999). Immunocytochemical detection and mapping of a Cytokeratin 18 neo- epitope exposed during early apoptosis. J Pathol 187, 567-572.

Performance Characteristics

Calibration:	The Units measured by the M30 CytoDeath™ ELISA are defined against a synthetic peptide containing the M30 and M6 epitopes. 1 U/L = 1.24 pM.
Working Range:	250 – 3 000 U/L.
Detection Limit:	60 U/L, Standard Z (0 U/L) + 3 S.D.
Reproducibility:	Intra-Assay (WA) Precision: CV < 7 % for values > 250 U/L. Inter-Assay (BA) Precision: CV < 10 % for values > 250 U/L.
Spike Recovery:	80–120 %
Linearity/Dilution:	80–120 %
Hook Effect:	No high dose "hook effect" occurs before 26 000 U/L which is well above concentrations of K18Asp396-NE (M30)-reactive material observed in cell culture samples.

Reagents

Coated Microstrips:	One Microplate, 96 dry wells (12 strips × 8 wells). The wells are coated with mouse monoclonal K18 anti-body M6.
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:	The Standards are Standard Zero (0 U/L), Low (250 U/L), Medium (1 000 U/L) and High (3 000 U/L).
TMB Substrate:	One vial containing 22 mL of TMB (3,3',5,5'-Tetramethylbenzidine) Solution.
Stop Solution:	One vial containing 6 mL of 1.0 M sulfuric acid.
Wash Tablet:	One tablet for preparation of 500 mL Wash Solution.

Assay Advantages

Easy to use:

All reagents are ready-to-use except for the M30-HRP Conjugate concentrate and the Wash Tablet.

Only few steps and a limited hands-on timeare required. Results are recorded as absorbance at 450nm using standard 96 well plate readers and are quantified against a standard.

Unique application range:

Coated Microstrips: One Microplate, 96 dry wells (12 strips × 8 wells). The wells are coated with mouse monoclonal K18 anti- body M6. antibody

Detecting specifically apoptosis, M30 CytoDeath™ ELISA does not reflect cellular metabolic activity often measured by viability tests as this may also be compromised during drug-induced cell cycle arrest, senescence or differentiation.

The assay shows a unique application to selectively detect apoptotic K18-positive target cells (e.g. carcinoma) in co-cultures with cells that are negative for K18 such as fibroblasts, endothelial cells and macrophages (e.g. stroma) or 3D tissue/tumor cultures and multi-cellular tumor spheres.

PEVIVA Products from VLVbio

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	Prod. No. 10800
Orange	Prod. No. 10850

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VLVbio

Vivalavida AB
172 66 Sundbyberg
Sweden
info@vlvbio.com
www.vlvbio.com

Intertek

bioaxxess

BIOAXXESS
Basepoint Business Centre
GL20 8SD Tewkesbury
United Kingdom

Your Partner in UK

Phone +44 (0) 1684 851 270
Fax +44 (0) 1684 851 272
Email info@bioaxxess.com
Web www.bioaxxess.co.uk