

M30 CytoDeath™ ELISA

Apoptosis detection in cell cultures

Catalog Prod. No. 10900

For research and laboratory use only.
Not for human or diagnostic use.

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:	<p>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.</p> <p>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.</p>
Samples:	Cell lysates or culture supernatants. <i>Not</i> 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:	<ul style="list-style-type: none">■ Zhang L, <i>et al.</i> (2010). Preclinical and clinical estimates of a cancer's basal apoptotic rate predict for the amount of apoptosis induced by subsequent pro-apoptotic stimuli. <i>Clin Cancer Res.</i> 16: 4478–89.■ Fayad W, <i>et al.</i> (2009). Identification of a novel topoisomerase inhibitor effective in cells overexpressing drug efflux transporters. <i>PLoS One</i> 4(10):e7238.■ Hernlund E, <i>et al.</i> (2009). Ovarian carcinoma cells with low levels of b-F1-ATPase are sensitive to combined platinum and 2-deoxy-D-glucose treatment. <i>Mol Cancer Ther</i> 8(7).■ Herrmann R, <i>et al.</i> (2008). Screening for Compounds that Induce Apoptosis of Cancer Cells Grown as Multicellular Spheroids. <i>J Biomol Screen.</i> 13(1):1–8.■ Lakshmikanthan V, <i>et al.</i> (2006). SAHA-sensitized prostate cancer cells to TNFalpha-related apoptosis-inducing ligand (TRAIL): mechanisms leading to synergistic apoptosis. <i>Int J Cancer</i> 119:221–8.■ Erdal H, <i>et al.</i> (2005). Induction of lysosomal membrane permeabilization by compounds that activate p53-independent apoptosis. <i>Proc. Natl. Acad. Sci. USA</i> 102, 192–197.■ Schutte B, <i>et al.</i> (2004). Keratin 8/18 breakdown and reorganization during apoptosis. <i>Exp Cell Res.</i> 297, 11–26.■ Kramer G, <i>et al.</i> (2004). Differentiation between Cell Death Modes using Measurements of Different Soluble Forms of Extracellular Cytokeratin 18. <i>Cancer Research</i> 64, 1751–1756.■ Hägg, M. <i>et al.</i> (2002). A novel high-through-put assay for screening of pro-apoptotic drugs. <i>Invest. New Drugs</i>, 20: 253-259.■ Leers MP, <i>et al.</i> (1999). Immunocytochemical detection and mapping of a Cytokeratin 18 neo-epitope exposed during early apoptosis. <i>J Pathol</i> 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” occur before 26 000 U/L which is well above concentrations of K18Asp396-NE (M30)-reactive material observed in cell culture samples.

Reagents

Coated Microplate:	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 a minimal number of steps are required. Results are recorded as absorbance at 450 nm using standard 96 well plate readers.
Broad application range:	The assay can be used to determine the effects of inhibitors or siRNA on apoptosis and offers a unique possibility of measuring apoptosis in multicellular spheroids. Suitable for drug screening, examination of time-kinetics and dose-response relationships.

Products from Peviva

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	

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