Validated Biomarker Assays Specific for Epithelial Cell Death

上皮细胞死亡的特异性生物标记物检测试剂盒

The M30-Apoptosense® and M65® ELISA assays discriminate between different modes of epithelial cell death *in vivo* by quantitative measurement of either released caspase-cleaved fragments of cytokeratin 18 (ccCK18/CK18F/CK18Asp396-NE/M30) or full-length CK18 intermediate filament protein analyzed in biological fluids such as blood specimen.

The M30-Apoptosense ELISA assay utilizes the M5 capture antibody and the M30 CytoDEATH™ antibody to detect CK18 fragment containing neoepitopes (NE) at positions aa 387-396, which are generated during the early stages of apoptosis by the lethal activation of caspases-3, -7 and -9.

The M65 ELISA also detects cleaved CK18 fragments, however, it uses a different detection antibody from M30, namely M5, that does not distinguish between the full-length protein and its fragments. Thus, the M65 ELISA measures both caspase-cleavage (indicative of apoptosis) and cellular release of intact CK18 (necrosis).

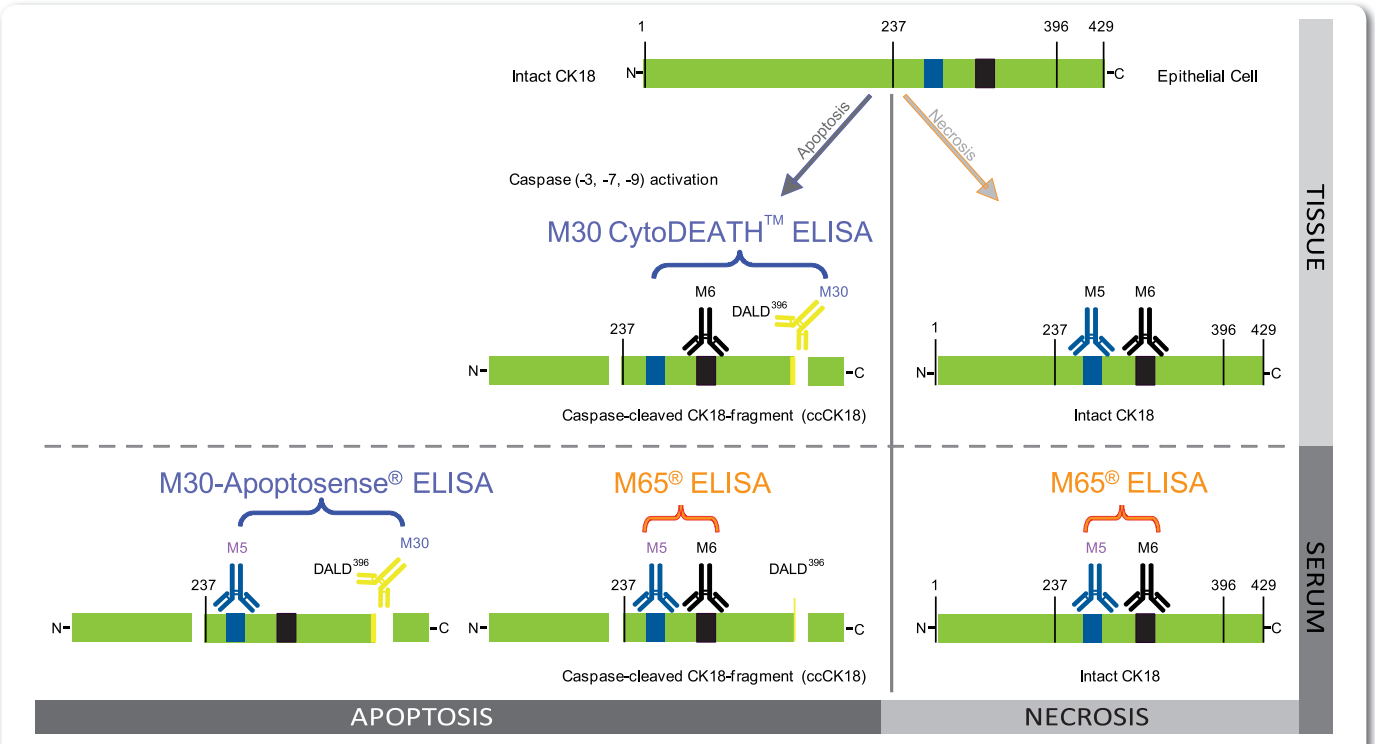
Since both assays utilize the same recombinant CK18 protein fragment as a reference, the ratio of M65/M30 potentially becomes a readout, which provides additional information on the predominant mode of (tumor) cell death. These assays have been used in multiple pre-clinical and clinical research applications, but increasingly, are being used as exploratory pharmacodynamic endpoints in clinical trials with apoptosis-modulating therapies.

During apoptosis the NE-containing CK18 fragment can be detected within stable cytokeratin complexes in serum and plasma, whereas during necrosis only soluble intact CK18 released from dead epithelial cancer cells can be detected. Recent studies suggest that apoptosis is not the sole death mode of successfully targeted tumors and that it is therefore important to monitor specifically both apoptotic and necrotic carcinoma cell death during cancer treatment.

Beyond cancer research, quantification of epithelial apoptotic cell death plays an emerging role in other pathological conditions like sepsis and liver damage through infection by hepatitis virus C. Note: physiological apoptosis of epithelial cells, e.g. intestinal epithelial turnover, does not lead to significant increase of detectable ccCK18 reactivity in serum or plasma from healthy individuals.

Caspase-cleaved Cytokeratin 18 (ccCK18/CK18F/CK18Asp396-NE/M30) A Validated Biomarker Specific for Epithelial Apoptosis

- Selective for epithelial cells and tissues (e.g. carcinoma cell apoptosis)
- Upon its cellular release accumulates in stable protein complexes in the circulation. Generates a persistent apoptotic signature with good biostability accessible for serial blood sampling
- Convergent measure of apoptosis mediated by multiple caspases (not limited to caspase-3)
- Can be measured by standard technologies: FC, ELISA, IHC, ICC and WB
- Specific for apoptosis (detects *lethal* caspase activation; no false piostive signal during proliferation/differentiation/inflammation)



	Apoptosis	Necrosis	Cell Lysate	Cell Culture Supernatant	Serum/Plasma
M30 CytoDEATH™ ELISA	✓	x	✓	✓	x
M30-Apoptosense® ELISA	✓	x	✓	✓	✓
M65® ELISA	✓	✓	x	✓	✓

M30 CytoDeath™ ELISA kit

Prod. No. #10900 — 1 Kit

For the quantitative measurement of epithelial cell apoptosis *in vitro* or toxicity studies using 3D-cell culture supernatants or lysates from human organoid (e. g. hepatocytes), spheroids (cancer stem cells) or primary (tumor biopsy) tissue section cultures. Designed as a high-throughput assay for functional screening and characterization of pro-apoptotic drugs using cell culture supernatants, and spheroid or tissue lysates.

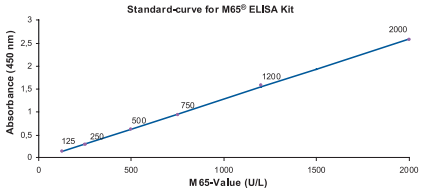
Species Reactivity	Human, monkey and bovine
Sample Type	Cell lysates or culture supernatants from CK18 positive (epithelial) apoptotic cells or tissues. Not suitable for serum or plasma samples.
Sensitivity	60 U/l, Standard Z (0 U/l) + 3 S.D.
Range	250 – 3,000 U/l
Detects	Soluble caspase-cleaved CK18 (ccCK18/CK18F/CK18Asp396-NE/M30)
Benefits	• Features a broader detection range specifically adapted for cellular screening systems • Simple - no special sample/reaction buffer or standardised assay conditions required • Flexible - accepts previously frozen cell lysates without loss of signal intensity • Minimal hands-on time (<60 mins) with ready-to-use reagents and pre-coated ELISA plate • Convenient 96-well microtiter plate • Suitable for automation

LIT: Identification of a Novel Topoisomerase Inhibitor Effective in Cells Overexpressing Drug Efflux Transporters: W. Fayad, et al.; PLoS ONE **4,** e7238 (2009) ▪ Conditional drug screening shows that mitotic inhibitors induce AKT/PKB-insensitive apoptosis: M. Berndtsson, et al.; J. Chem. Biol. 2, 81 (2009) ▪ **For a comprehensive bibliography please visit our website.**

M65® ELISA kit (CE-Mark)

Prod. No. #10020 1 Kit

For the quantitative measurement of total cell death (apoptosis and necrosis) *in vitro* and *in vivo*. In combination with the M30-Apoptosense® assay (which specifically measures apoptosis) the M65 ELI - SA kit is useful for the assessment of the relative contribution of apoptosis towards the overall/total epithelial cell death.



Species Reactivity	Human
Sample Type	Serum or plasma (EDTA, citrate, heparin plasma) samples containing CK18 released from dead epithelial cells. Culture supernatants from CK18 positive dead cells or tissues.
Sensitivity	11 U/l, Standard A (0 U/L) + 2 S.D.
Range	125 – 2 000 U/l
Detects	Intact CK18 and soluble caspase-cleaved CK18 (ccCK18/CK18F/CK18Asp396-NE/M30)
Benefits	• Quality assurance of certified (CE-marked) *in vitro* diagnostic (IVD) device* • Independent, robust validation (GCLP) qualifies M65 ® ELISA as fit-for-purpose total epithelial cell death biomarker • Minimal hands-on time (<60 mins) with ready-to-use reagents and pre-coated ELISA plate • Convenient 96-well microtiter plate (flexible with 12 separable 8-well strips) • Suitable for automation

* For laboratory and research use only in USA, Canada or Japan. Not for diagnostic use in USA, Canada or Japan.

M30-Apoptosense® ELISA kit (CE-Mark)

1 Kit

For the quantitative measurement of epithelial cell apoptosis *in vitro* and *in vivo*. Can be used for the quantitative and specific measurement of human tu- mor xenograft apoptosis in mouse models using plasma samples.

Species Reactivity	Human
Sample Type	Serum or plasma (EDTA, citrate, heparin plasma) samples containing caspase-cleaved CK18 from apoptotic epithelial cells. Cell lysates or culture supernatants from CK18 positive (epithelial) apoptotic cells or tissues.
Sensitivity	25 U/l, Standard A (0 U/l) + 2 S.D.
Range	75 – 1 000 U/l
Detects	Soluble caspase-cleaved CK18 (ccCK18/CK18F/CK18Asp396-NE/M30)
Benefits	• Quality assurance of certified (CE-marked) *in vitro* diagnostic (IVD) device* • Independent, robust validation (GCLP) qualifies M30-Apoptosense® ELISA as fit-for-purpose epithelial apoptosis biomarker • Two quality controls included • Minimal hands-on time (<60 mins) with ready-to-use reagents and pre-coated ELISA plate • Convenient 96-well microtiter plate (flexible with 12 separable 8-well strips) • Suitable for automation

* For laboratory and research use only in USA, Canada or Japan. Not for diagnostic use in USA, Canada or Japan.

Literature References M30-Apoptosense®ELISA/M65®ELISA:

Cancer Research

Differentiation between cell death modes using measurements of different soluble forms of extracellular cytokeratin 18: G. Kramer, et al.; Cancer Res. **64,**1751 (2004) ▪ Determining tumor apoptosis and necrosis in patient serum using cytokeratin 18 as a biomarker: S. Linder, et al.; Cancer Lett. **214,**1 (2004) ▪ Apoptosis pathway-targeted drugs--from the bench to the clinic: J. Cummings, et al; Biochim. Biophys. Acta **1705,**53 (2004) ▪ Induction of lysosomal membrane permeabilization by compounds that activate p53-independent apoptosis: H. Erdal, et al.; PNAS **102,**192 (2005) ▪ Validation of pharmacodynamic assays to evaluate the clinical efficacy of an antisense compound (AEG 35156) targeted to the X-linked inhibitor of apoptosis protein XIAP: J. Cummings, et al.; Br. J. Cancer **92,** 532 (2005) ▪ Apoptosis in a tissue like culture model of human colorectal adenoma cells: K. Leuhuber, et al.; Tissue Cell **238,**203 (2006) ▪ Sense and sensibility: the use of cell death biomarker assays in high throughput anticancer drug screening and monitoring treatment responses: M. Shoshan, et al.; Expert Opin. Drug Discov. **1,** 585 (2006) ▪ Screening for Compounds that Induce Apoptosis of Cancer Cells Grown as Multicellular Spheroids: R. Herrmann, et al.; J. Biomol. Screen. **13,** 1 (2007) ▪ Specific demonstration of drug-induced tumour cell apoptosis in human xenografts models using a plasma biomarker: M.H. Olofsson, et al.; Cancer Biomark. **5,** 117 (2009) ▪ Docetaxel induces apoptosis in hormone refractory prostate carcinomas during multiple treatment cycles: G. Kramer, et al.; Br. J. Cancer **94,** 1592 (2006) ▪ Cytokeratin 18 is a useful serum biomarker for early determination of response of breast carcinomas to chemotherapy: M.H. Olofsson, et al.; Clin. Cancer Res. **13,**3198 (2007) ▪ Evaluation of circulating tumor cells and serological cell death biomarkers in small cell lung cancer patients undergoing chemotherapy: J.M. Hou, et al.; Am. J. Pathol. **175,**808 (2009)

Liver Disease (NAFLD/NASH/HCV/HBV/ALF/HCC) Research

Soluble forms of extracellular cytokeratin 18 may differentiate simple steatosis from nonalcoholic steatohepatitis: Y. Yilmaz, et al.; World J. Gastroenterol. **13,**837 (2007) ▪ Noninvasive diagnosis of non- alcoholic fatty liver disease using serum biomarkers: R.R. Mitry, et al.; Hepatology **46,**2047 (2007) ▪ A Novel Diagnostic Biomarker Panel for Obesity related Nonalcoholic Steatohepatitis (NASH): Z.M. Younossi, et al.; Obes. Surg. **18,**1430 (2008) ▪ Cytokeratin 18, a marker of cell death, is increased in children with suspected nonalcoholic fatty liver disease: M.B. Vos, et al.; J. Pediatr. Gastroenterol. Nutr. **47,**481(2008) ▪ The clinical utility of biomarkers and the nonalcoholic steatohepatitis CRN liver biopsy scoring system in patients with nonalcoholic fatty liver disease: R. Malik, et al.; J. Gastroen- terol. Hepatol. **24,**564 (2009) ▪ Cytokeratin-18 fragment levels as noninvasive biomarker for nonalcoholic steatohepatitis: A multicenter validation study: A. Feldstein, et al.; Hepatology, **50,**1072 (2009) ▪ Effects of ursodeoxycholic acid in combination with vitamin E on adipokines and apoptosis in patients with nonalcoholic steatohepatitis: M.L. Balmer, et al.; Liver Int. **29,**1184 (2009)

Sepsis Research

Elevated serum levels of epithelial cell apoptosis-specific cytokeratin 18 neoepitope M30 in critically ill patients: G.A. Roth, et al.; Shock **22,**218 (2004) ▪ Cell death serum biomarkers are early predic- tors for survival in severe septic patients with hepatic dysfunction: S. Hofer, et al.; Critical Care **13,**R93 (2009)

For a comprehensive bibliography please visit our website.

Purified (PF) = Purified (Preservative free); FC = Flow Cytometry; ICC = Immunocytochemistry; IP = Immunoprecipitation; IHC = Immunohistochemistry (FS = Frozen Sections, PS = Paraffin Sections); WB = Western blot; BP = Blocking Peptide

PEVIVA

M30 CytoDEATH™,mAb – The Gold Standard for the Detection of Epithelial Apoptosis

Unlabeled 200 tests

Benefits	Features / Key Advantages
Apoptosisspecific	Recognizes the CK18-Asp^{396} neo-epitope (M30) on human, monkey and bovine caspase-cleaved CK18. Does not cross-react with intact CK18 within viable cells or CK18, which is released from necrotic epithelial cells.
Broad application range	The M30 CytoDEATH™ antibody has been successfully used in Westernblot, immunocytochemistry, flow cytometry and immunohistochemistry, including frozen and formalin-fixed, paraffin-embedded tissue sections.
Recommended for formalin-fixed paraffin-embeddedtissue	Recommended for routinely fixed tissue samples. Retrograde studies are possible,even on archive material, as the M30 antigen is abundant and formalin-resistant.
Flexible selection of sampling time point	Detects apoptosis earlier than Annexin V or anti-active caspase antibodies without losing signal strength at late (TUNEL-positive) stages of apoptosis.

M30 CytoDEATH™, mAb – Biotin Conjugate

Biotin 200 tests

Benefits	Features / Additional Advantages
Added convenience for immunohistochemistry	Two-step tool for the detection of apoptosis in epithelial cells by immunohistochemistry. No additional anti-mouse IgG biotin conjugated secondary antibodies required. No background problems especially with human xenograft tissue within mouse or rat samples.

M30 CytoDEATH™, mAb – Fluorochrome Conjugates

Fluorescein 200 tests
NEW! Orange 200 tests
NEW! Red 200 tests

Benefits	Features / Key Advantages
Added convenience for flow cytometry and immunocytochemistry	One-steptool for the detection of apoptosisin epithelial cells by flow cytometry and immunocytochemistry. No additional anti-mouse IgG fluorochrome-conjugated secondary antibodies required. Can be used for multiplexing e.g. analysing circulating tumor cells (CTC) or GFP-tagged cells.

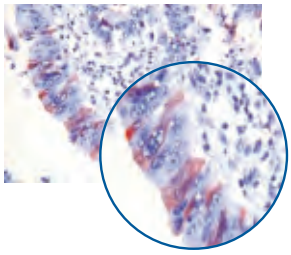


FIGURE: Detection of apoptosis in a formalin-fixed and paraffin-embedded tissue section from a human colon cancer showing confined cytoplasmic staining for ccCK18/CK18F using M30 CytoDEATH™, mAb (Prod.No.10700). Secondary detection with anti-mouse IgG-biotin, streptavidin-POD and AEC as substrate, counterstained with hematoxylin.

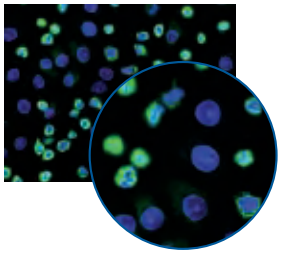


FIGURE:HeLa cells that received combined treatment with CHX and TRAIL show a sustained and strong caspase activity and concomitant ccCK18/CK18F accumulation as detected by the M30 CytoDEATH™ Fluorescein (Prod. No. 10800) antibody.

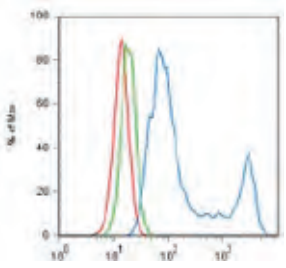


FIGURE: HeLa (human cervical cancer) cells were fixed in methanol and stained with M30 CytoDEATH™ Orange antibody (Prod. No. 10830). Blue line: Apoptotic HeLa cells (preincubated with CHX -10ug/ml for 1h- followed by rhsTRAIL -200ng/ml for 2h) were stained with M30 CytoDEATH™ Orange. Green line: Untreated, viable HeLa cells stained with M30 CytoDEATH™ Orange. Red line: Untreated, viable HeLa cells were left unstained.

LIT: Immunocytochemical detection and mapping of a cytokeratin 18 neo-epitope exposed during early apoptosis: M.P. Leers, et al.; J. Pathol. **187,**567 (1999) ▪ Apoptosis of circulating tumor cells in prostate cancer patients: C. J. Larson, et al.; Cytometry A. **62,**46 (2004) ▪ Uncovering the roles of intermediate filaments in apoptosis: N. Marceau; Methods Cell Biol. **78,**95 (2004) ▪ Apoptosis resistance in Barrett's esophagus: ex vivo bioassay of live stressed tissues: K. Dvorakova, et al.; Am. J. Gastroenterol. **100,**424 (2005) ▪ Oncosis represents the main type of cell death in mouse models of cholestasis: P. Fickert, et al.; J. Hepatol. **42,**378 (2005) ▪ Assessment of apoptosis by immunohistochemical markers compared to cellular morphology in ex vivo-stressed colonic mucosa: H. Holubec, et al.; J. Histochem. Cytochem. **53,**229 (2005) ▪ Expression levels of Akt in nimesulide-treated squamous carcinoma cell lines of the head and neck: L. Vormittag, et al.; Oncol. Rep. **13,**207 (2005) ▪ Specific demonstration of drug-induced tumour cell apoptosis in human xenografts models using a plasma biomarker: M.H. Olofsson, et al.; Cancer Biomark. **5,**117 (2009)▪ **For a comprehensive bibliography please visit our website.**

Related Products

Product	Isotype	Specificity	Application	Prod. No.	Size
Cytokeratin 18 (human), mAb (M5)	Mouse IgG2b	Human	FC, ICC, IHC (FS, PS), IP, WB	10600	200 tests
Cytokeratin 18, mAb (M6)	Mouse IgG2a	Human, mouse, rat and dog	FC, ICC, IHC (FS, PS), IP, WB	10650	200 tests

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