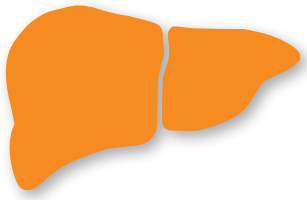
PEVIVA

diapharma®

M30 Apoptosense® ELISA and M65® ELISA

Biomarker Assays for NASH Research



For Research Use Only in the US and Canada

NASH – A Global Disease

Non-Alcoholic Fatty Liver Disease (NAFLD) is the most common liver disease in Western countries. It is strongly connected to the epidemic increase of obesity and type 2 diabetes.

Terminology

NAFLD
Covers the entire spectrum of fatty liver disease in individuals without significant alcohol consumption or viral infection, ranging from simple steatosis (fatty liver) to steatohepatitis and cirrhosis.

NAFL
The early form of NAFLD. Characterized by the presence of hepatic steatosis without inflammation.

NASH
The more progressive form of NAFLD. Characterized by the presence of hepatic steatosis, inflammation, and cell injury, with or without fibrosis.

Over the last two decades, the incidence of NAFLD has increased dramatically and the disease affects a considerable share of today's global population. NAFLD refers to a spectrum of conditions that are characterized by excessive accumulation of fat in the liver. The early form of NAFLD, Non-Alcoholic Fatty Liver (NAFL), is described as the presence of simple steatosis in the liver. In Non-Alcoholic steatohepatitis (NASH), a more progressive and form of NAFLD, steatosis is accompanied by inflammation and hepatocyte injury, with or without fibrosis. NAFLD can progress to cirrhosis, liver failure, and hepatocellular carcinoma (HCC), and it is associated with an increased risk for liver-related and cardiovascular mortality.

The prevalence of NAFLD worldwide is estimated to be 20–35%, and of those, 10–30% have NASH. Within the obese and diabetic population these percentages are much higher, sometimes reported to be as high as 75–95% and 40%, respectively. In the United States the incidence of this disease is rising exponentially. Approximately 65 million Americans currently have NAFLD and 16.5 million have NASH. By 2030, these numbers are expected to increase to 100 million for NAFLD, and 27 million for NASH. Even more alarming, 10% of the pediatric population in the US is thought to have NAFLD.

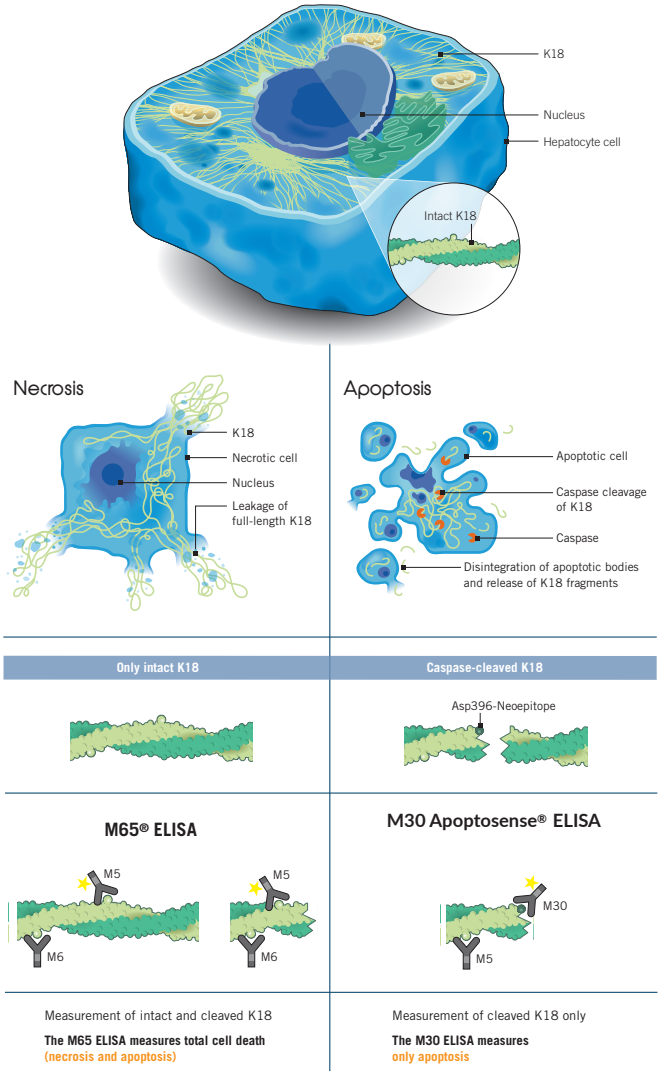
Reliable Tools for NASH Research Are Vital

Common Methods for Measuring NASH

The standard method for classification of NASH is the histological analysis of a liver biopsy sample. Liver biopsy is a costly and invasive procedure that is associated with risks and discomfort for the subject. Furthermore, liver biopsies are prone to a high rate of sampling error that can lead to misclassification of up to one-fourth of all subjects. It is also widely recognized that measuring serum aminotransferase levels, another standard method for assessing liver diseases, is not useful for the detection of NASH. Reliable noninvasive tools that can identify subjects with NASH are vital.

The Role of Keratin 18 in NASH

When simple steatosis in NAFLD is accompanied by inflammation and cell injury, the disease is described as NASH. In the damaged liver, cell death occurs primarily by necrosis or apoptosis (programmed cell death). Apoptosis is a characteristic feature of liver disease, and it may be one of the drivers involved in the progression of NAFLD. Early in apoptosis of hepatocyte cells, caspases are activated which cleave a number of substrates, including the intermediate filament keratin 18 (K18) resulting in K18 fragments (cK18). During necrosis, hepatocytes lose their cell membrane integrity and intact K18 is released. Hepatocyte ballooning, which is a major histological feature of NASH, is associated with the loss of K18 immunostaining in the cytoplasm. During liver injury, both intact K18 and cK18 can be detected in the blood.



M30 Apoptosense® ELISA and M65® ELISA

Reliable Noninvasive Tools for NASH Research

The M30 Apoptosense® ELISA and M65® ELISA are robust and reliable tools to measure the concentration of keratin (K18) and its caspase-cleaved fragments (cK18) in NASH research studies. The M30 Apoptosense® ELISA specifically detects cK18, indicative of apoptosis, whereas the M65® ELISA detects both intact K18 and cK18, indicative of total cell death (apoptosis and necrosis). Increased serum levels of both cK18 and K18 are associated with ballooning, as well as steatosis, lobular inflammation, and fibrosis, and both K18 and cK18 have shown stronger positive correlations with the composite NAFLD Activity Score (NAS) than serum aspartate and alanine aminotransferase (AST and ALT). Furthermore, decreased levels of cK18 have been shown to correlate with an improvement in histology in response to treatment in at least two published randomized control NASH trials.

K18 and cK18 in NASH Drug Development

K18 and cK18 as Secondary Endpoints in NASH Clinical Trials

Liver biopsy is still considered the "gold standard" for diagnosis of NASH, and histological endpoints are currently required by the FDA for approval of NASH drugs. However, because of the many drawbacks of liver biopsy, noninvasive biomarkers are urgently needed not only to identify subjects with NASH, but also to determine their response to novel therapeutic agents.

An AASLD-FDA Joint Workshop has recommended a decrease in markers of hepatic injury or cell death, such as K18 and cK18, as a secondary endpoint for early phase NASH trials. These early phase studies are generally of limited duration (12-18 months), and histological endpoints may be difficult to meet in this time frame. In a subject with NASH, a decline in K18 and cK18 levels in response to a pharmacologic intervention may reflect a decrease in active apoptosis associated with reduced fibrosis and inflammation. Therefore, K18 and cK18 may be useful as surrogate markers to assess efficacy of a drug, and they may provide important information for making decisions on whether to move forward with additional trials.

K18 and cK18 Assays as Prescreening Tools in NASH Clinical Trials

One of the biggest challenges facing pharmaceutical companies involved in NASH clinical trials is the cost-effective and timely enrollment of study subjects. Despite the high prevalence of NAFLD, there is an overall low awareness of this disease among health care providers and in the general population. As a result, even subjects who have several of the risk factors that are linked to NASH - and they therefore have a high probability of having NASH - are frequently not referred to specialists who are involved in clinical trials. Furthermore, recruited subjects are often required to undergo a liver biopsy screening to verify that they are eligible to enroll in a study. Because many of the screened subjects do not meet the histological inclusion criteria, the number of unproductive procedures adds to the cost and duration of these clinical trials.

A strategy to reduce the screen failure rate is to prescreen recruited subjects with serum biomarkers or imaging tests prior to performing a biopsy, with the goal to enrich the study population that meets the required features for enrollment. A ballooning score of ≥1 is one of the inclusion criteria for most NASH clinical trials. Up to 40% of the NAFLD population do not display any ballooning (ballooning score 0) by biopsy, which makes these individuals ineligible for enrollment. Ballooning is associated with elevated levels of K18 and cK18 in serum. Circulating K18 and cK18 may thus be useful as noninvasive markers for hepatocyte ballooning, and therefore be helpful for reducing the screen failure rate in NASH clinical trials.

M30 Apoptosense® ELISA
(Prod. No P10011)

The M30 Apoptosense® ELISA measures the concentration of caspase-cleaved K18 (cK18) in human plasma, serum or cell culture, reflecting the level of apoptosis. The assay is based on the unique M30 antibody, which recognizes a neo-epitope of K18 formed after caspase cleavage. The assay can be combined with the M65® ELISA for the analysis of cell death mode (apoptosis or necrosis).

The M30 Apoptosense® ELISA measures the level of hepatocyte apoptosis in research subjects with liver diseases, e.g. NASH, Alcoholic Hepatitis (AH), Hepatitis C virus infection (HCV) and more.

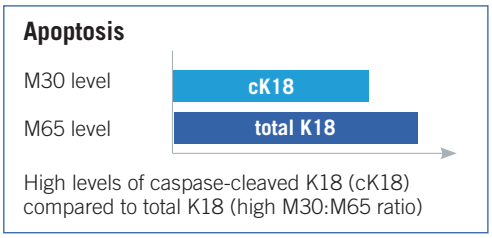
Features of the M30 Apoptosense® ELISA

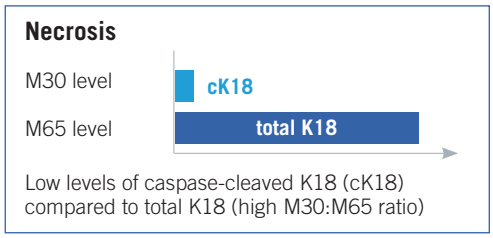
- Specific measurement tool for apoptosis quantification in K18 positive cells
- Suitable to use together with the M65® ELISA for quantification of apoptosis, necrosis and total cell death
- Sandwich ELISA with a 96-well plate in a convenient ready-to-use format
- Can be split up for use at several occasions

The M30 Apoptosense® ELISA is For Research Use Only in the US and Canada.

M30:M65 Ratios Indicate Cell Death Mode

The ratios between the M30 Apoptosense® ELISA (measuring caspase-cleaved K18, cK18) and the M65® ELISA (measuring total K18) reflect the cell death mode. The M30:M65 ratio is assesed by comparing the amount of apoptosis (M30) to the amount of total cell death (M65). High M30:M65 ratios indicate that the cell death is mainly due to apoptosis. In contrast, low M30:M65 ratios suggest necrosis is the predominant cause of cell death.





M65® ELISA
(Prod. No P10020)

The M65® ELISA measures soluble K18 released from dying cells. It can be used to assess overall cell death, due to apoptosis and necrosis. The M65® ELISA is intended for human serum or plasma.

The M65® ELISA is primarily intended to be used together with the M30 Apoptosense® ELISA. When used together, the quantification of total cell death, apoptosis and necrosis is possible. As both assays are calibrated against the identical reference, the combination of the M30 Apoptosense® ELISA and the M65® ELISA allows determination of the relative contribution of apoptosis to total cell death. All reagents are provided in a convenient ready-to-use format.

How To Order

VLVbio is collaborating with distributors all around the world to provide fast, reliable and convenient service. In the US and Canada, please visit Diapharma on the web at **diapharma.com** or e-mail **info@diapharma.com.** You can also e-mail VLVbio directly at **marketing@vlvbio.com.**

PEVIVA ELISA kits – For detection of NASH

ELISA Products	Prod. No	Apoptosis	Necrosis
M30 Apoptosense® ELISA	P10011	✔	✖
M65® ELISA	P10020	✔	✔

Other PEVIVA Line Products

ELISA Products	Prod. No
M30 CytoDeath™ ELISA	P10900
M65 EpiDeath® ELISA	P10040
Monoclonal Antibody Products	**Prod. No**
M5 Keratin 18 mAb	P10600
M6 Keratin 18 mAb	P10650
M30 CytoDEATH™ mAb (unlabelled)	P10700
M30 CytoDEATH™ mAb Biotin	P10750
M30 CytoDEATH™ mAb Fluorescein	P10800
M30 CytoDEATH™ mAb Orange	P10830

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