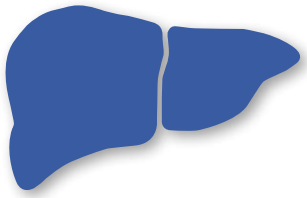
PEVIVA



M30 Apoptosense ELISA

A biomarker assay for detection and screening of NASH

NASH – A Global Disease

In the Western countries, Non-Alcoholic Fatty Liver Disease (NAFLD) is the most common liver disease, strongly connected to the epidemic increase of obesity and type 2 diabetes.[1]

Terminology

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

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

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

Non-Alcoholic Steatohepatitis (NASH), a more progressive and serious form of NAFLD, has during the last couple of decades become the most common cause of liver disease globally and a public health problem. The number of affected patients is growing rapidly and the disease affects a considerable share of today's global population. The incidence of NAFLD worldwide is reported to be around **20–35%**, and of these around **10–30%** have NASH. Within the obese and diabetic population, the number of NAFLD and NASH patients is much higher, sometimes reported to be as high as **75–95%** and **40%** respectively.[1,2,3]

It is projected that NAFLD and NASH will become the major cause of liver related morbidity and mortality during the next decade. The early form of NAFLD, Non-Alcoholic Fatty Liver (NAFL), is described as the presence of simple steatosis in the liver. NASH, the more progressive form, is characterized as simple steatosis accompanied by inflammation, cell injury and ballooning, with or without fibrosis. It can progress to cirrhosis, liver failure and hepatocellular carcinoma (HCC), and is associated with dramatically increased liver-related and cardiovascular mortality.[1, 2, 3, 4]

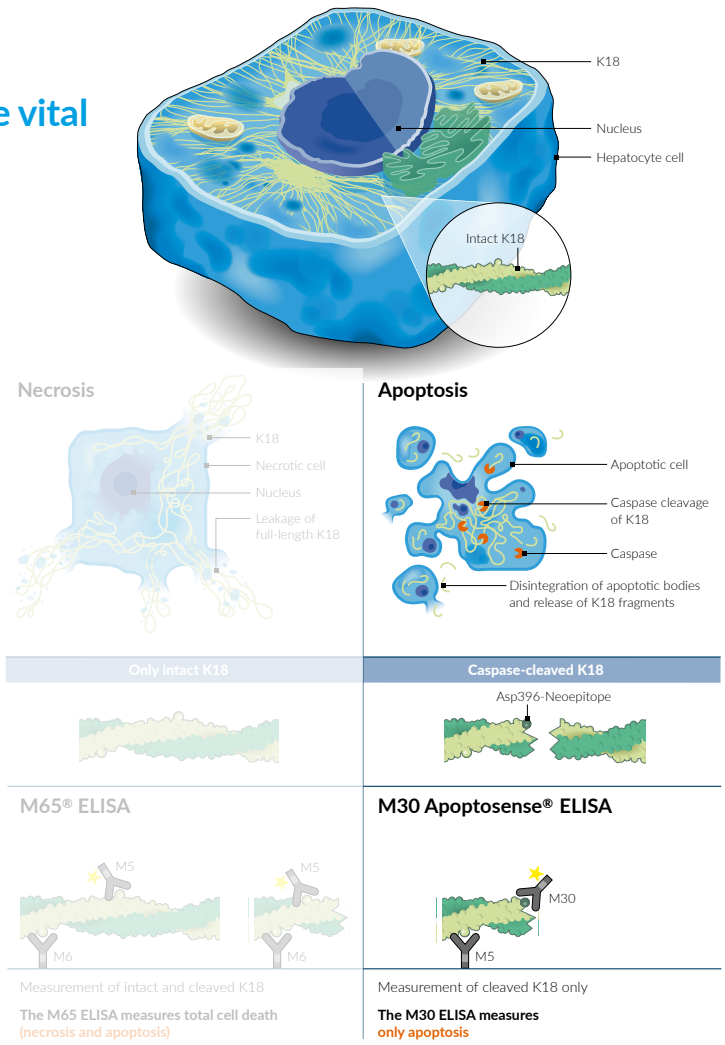
Reliable tools for detection of NASH are vital

Common methods for detection of NASH

The standard method for diagnosing and staging NASH has been through liver biopsy. This is a costly and invasive method associated with risks and discomfort for the patient, as well as misdiagnosis in up to one fourth of all patients.[9,10] It is also widely recognized that aminotransferases (collected in blood), another standard method for detection of liver diseases, are not reliable in the identification of NASH.[9,11] Therefore it is recommended that physicians should use additional tools to facilitate the identification of patients at risk for NASH.[10] Reliable non-invasive techniques that can diagnose and monitor patients with NASH are vital.[6,12]

The role of keratin 18 in NASH

When simple steatosis in NAFLD is accompanied by inflammation of hepatocytes, the disease is described as NASH.[4] This prominent characteristic of the disease is mainly caused by hepatocyte cell death due to apoptosis.[5,6,7] Early on in the apoptosis of hepatocytes, caspases (a form of proteases) are activated and cleave the protein keratin 18 (K18), and the resulting fragments are subsequently released into the blood.[8] These K18 fragments can be efficiently quantified by the unique M30 Apoptosense® ELISA.[6]

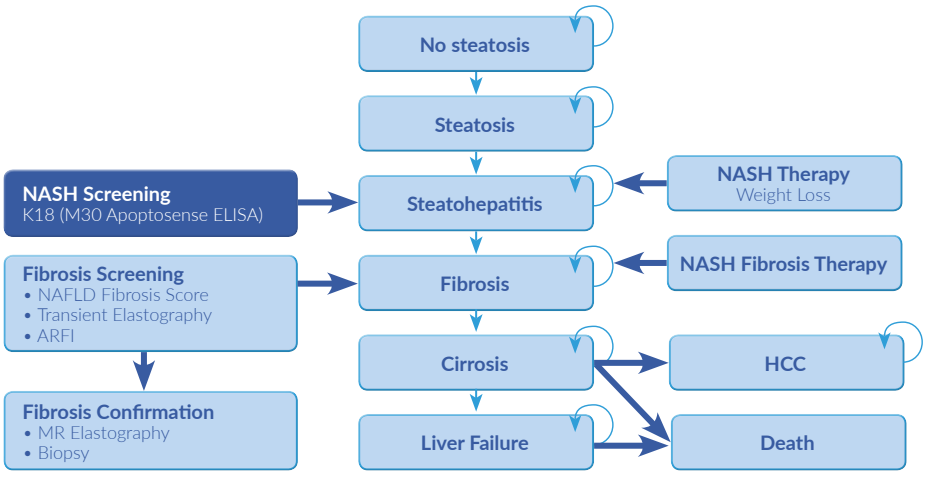


M30 Apoptosense® ELISA

A reliable non-invasive tool for the detection and screening of NASH

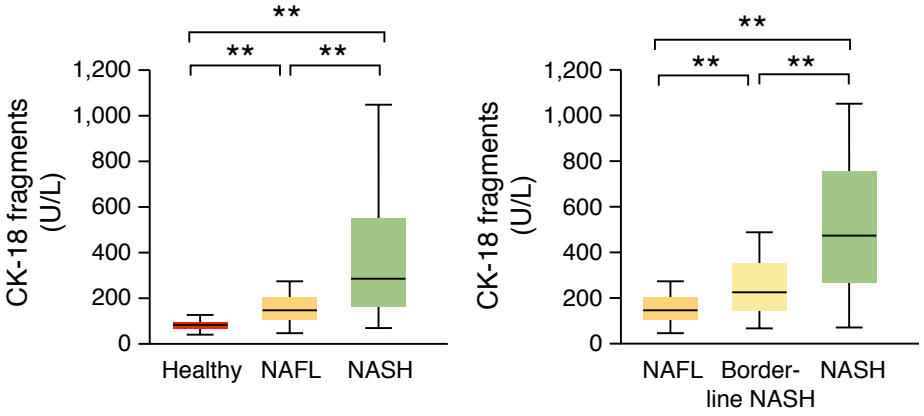
The M30 Apoptosense® ELISA measures the concentration of K18 fragments and is a specific and reliable tool for the detection and screening of NASH. Two recent meta-analyses, Musso *et al.* and Chen *et al.* demonstrated that levels of K18 fragments predict the presence of NASH with a pooled AUROC of 0.82, 78 % sensitivity and 86 % specificity and AUROC of 0.8445, 83 % sensitivity and 71 % specificity, respectively.[7,13]

The model illustrates the natural history of NAFLD, screening strategies and therapies.



Adapted from Zhang *et al*, 2015. Markov model illustrating the natural history of NAFLD and screening strategies and therapies.

M30 levels in patients with NASH



Adapted from Bantel *et al.* Am J Gastro.2014

The figure shows that the M30 Apoptosense® ELISA allows the significant discrimination between NASH (n=74) and NAFL (n=23), as well as between NASH and healthy (n=23) ($p < 0.01$). Furthermore, the M30 Apoptosense® ELISA can distinguish between NAFL and borderline NASH (n=40) ($p < 0.01$).[11]

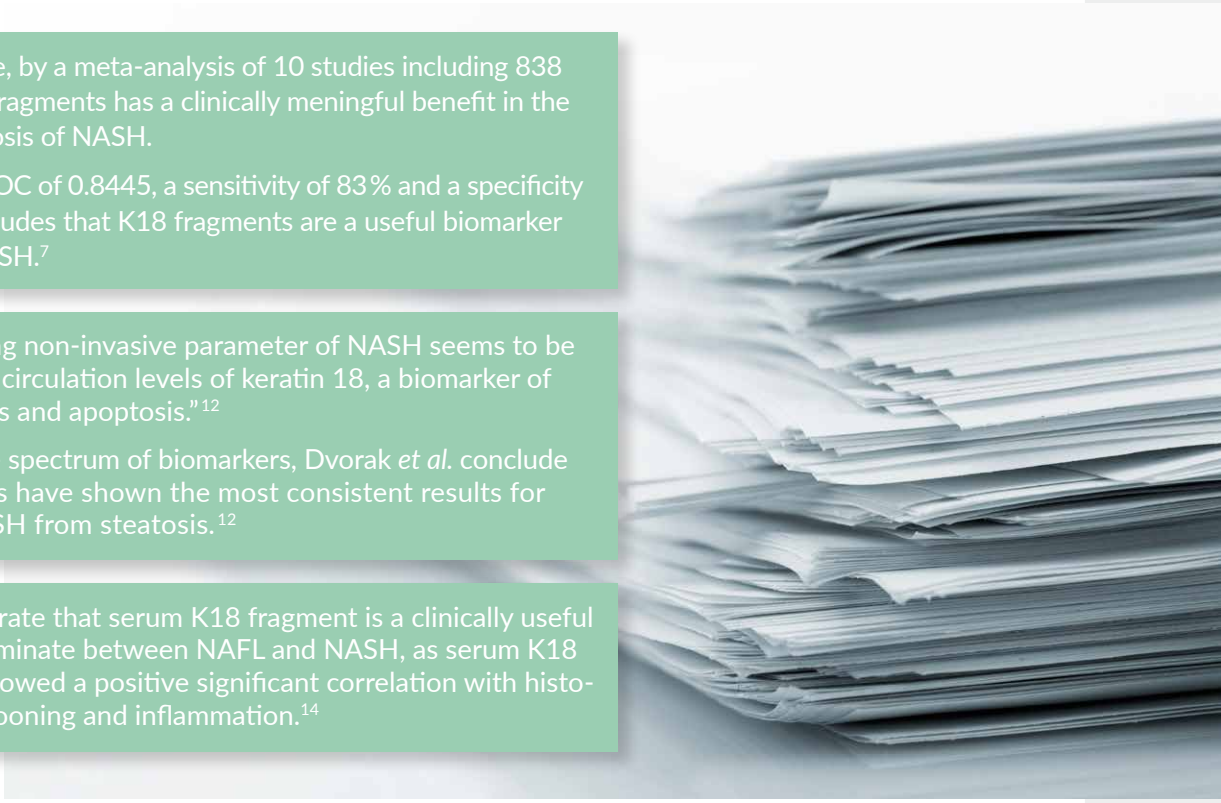
Chen *et al.* conclude, by a meta-analysis of 10 studies including 838 patients, that K18 fragments has a clinically meningful benefit in the non-invasive diagnosis of NASH.

With a pooled AUROC of 0.8445, a sensitivity of 83% and a specificity of 71%, Chen concludes that K18 fragments are a useful biomarker for screening of NASH.[7]

"The most promising non-invasive parameter of NASH seems to be the examination of circulation levels of keratin 18, a biomarker of hepatocyte necrosis and apoptosis."[12]

Looking into a large spectrum of biomarkers, Dvorak *et al.* conclude that K18 fragments have shown the most consistent results for differentiating NASH from steatosis.[12]

Aida *et al.* demonstrate that serum K18 fragment is a clinically useful biomarker to discriminate between NAFL and NASH, as serum K18 fragments levels showed a positive significant correlation with histologic steatosis, ballooning and inflammation.[14]



M30 Apoptosense® ELISA in pediatric NAFLD

Feldstein *et al.* demonstrate that levels of K18 fragments in plasma are significantly higher in children with NASH compared to children with hepatic steatosis. They also demonstrate that K18 in combination with biopsy has excellent accuracy for diagnosing NASH, with an AUC of 0.933. Therefore, Feldstein *et al.* conclude that using K18 fragments as a marker of hepatocyte apoptosis is a reliable test to diagnose NASH in children with suspected NAFLD.[15] Fitzpatrick *et al.* also show that the level of K18 fragments as a marker correlates well with inflammation and that K18 is useful for stratifying disease severity in pediatric NAFLD.[16]

M30 Apoptosense® ELISA
(Prod. No 10011)

The M30 Apoptosense® ELISA measures the concentration of caspase-cleaved K18 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 patients with liver diseases, e.g. NASH, Alcoholic Hepatitis (AH), Hepatitis C virus infection (HCV) and more.

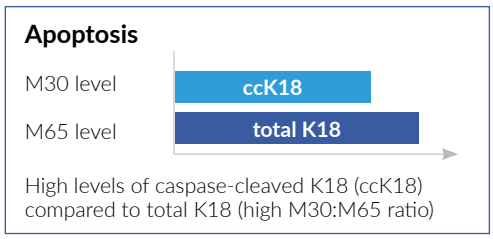
Features of the M30 Apoptosense® ELISA

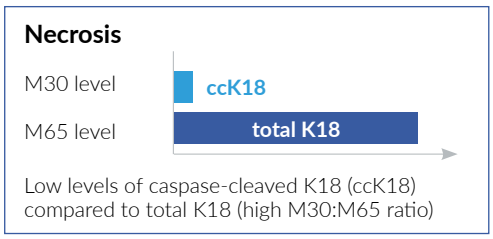
- Specific measurement tool for apoptosis quantification in K18 positive cells
- Suitable to use together with the M65® ELISA for quantification of apoptosis, necrosis and total cell death
- Sandwich ELISA with a 96-well plate in a convenient ready-to-use format
- Can be split up for use at several occasions

The M30 Apoptosense® ELISA is CE marked as a medical device for *in vitro* diagnostic use in Europe.

M30:M65 ratios indicate Cell Death Mode

The ratios between the M30 Apoptosense® ELISA (measuring caspase-cleaved K18) 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 10020)

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, and is CE marked as a medical device for *in vitro* diagnostic use in Europe.

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 for you. Please contact your local distributor, visit **www.vlvbio.com/distributors/** or e-mail VLVbio directly at **marketing@vlvbio.com**

PEVIVA ELISA kits – For detection of NASH			
ELISA Products	Prod. No	Apoptosis	Total cell death
M30 Apoptosense® ELISA	10011	✔	–
M65® ELISA	10020	–	✔

Other PEVIVA Line Products	
ELISA Products	Prod. No
M30 CytoDeath™ ELISA	10900
M65 EpiDeath® ELISA	10040
Monoclonal Antibody Products	Prod. No
M5 Keratin 18 mAb	10600
M6 Keratin 18 mAb	10650
M30 CytoDEATH™ mAb (unlabelled)	10700
M30 CytoDEATH™ mAb Biotin	10750
M30 CytoDEATH™ mAb Fluorescein	10800
M30 CytoDEATH™ mAb Orange	10830

REFERENCES

1 Zhang et al, 2015. Cost-utility analysis of nonalcoholic steatohepatitis screening. European Society of Radiology. 25(11), 3282-3294.
2 Golabi et al, 2015. Current complications and challenges in nonalcoholic steatohepatitis screening and diagnosis. Expert review of gastroenterology & hepatology. 10(1), 63-71.
3 LaBrecque et al, 2014. World Gastroenterology Organisation Global Guidelines: Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis. Journal of Clinical Gastroenterology. 48(6), 467-473.
4 Sakhuja et al, 2014. Pathology of alcoholic liver disease, can it be differentiated from nonalcoholic steatohepatitis? World Journal of Gastroenterology. 20(44), 16474-9.
5 Wieckoswka et al, 2006. In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease. Hepatology. 44(1), 27-33.
6 Shen et al, 2012. Assessment of non-alcoholic fatty liver disease using serum total cell death and apoptosis markers. Alimentary Pharmacology and Therapeutics. 36(11), 1057-9.
7 Chen J et al, 2014. Serum cytokeratin-18 in the diagnosis of non-alcoholic steatohepatitis: A meta-analysis. J, Hepatology research. 44(8),854-862.
8 Ku et al, 2016. Keratins: Biomarkers and Modulators of Apoptotic and Necrotic Cell Death in the Liver. Hepatology. [Epub ahead of print]
9 Machado, & Cortez-Pinto, 2013. Non-invasive diagnosis of non-alcoholic fatty liver disease. Journal of Hepatology, 58(5), 1007-10019.
10 Yilmaz et al, 2009. Cytokeratin-18 fragments and biomarkers of the metabolic syndrome in nonalcoholic steatohepatitis. World Journal of Gastroenterology, 15(35), 4387-4391.
11 Bantel et al, 2014. Robust detection of liver steatosis and staging of NAFLD by an improved ELISA for serum cytokeratin-18 fragments. Am J Gastro. 109(1):140-1.
12 Dvorak et al, 2014. Use of Non-Invasive Parameters of Non-Alcoholic Steatohepatitis and Liver Fibrosis in Dayly Practice – An Exploratory Case-Control Study. PLoS ONE. 9(10).
13 Musso et al, 2011. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive test for liver disease severity. Annals of Medicine. 43(8), 617-649.
14 Aida et al, 2014. Serum cytokeratin 18 fragment level as a noninvasive biomarker for non-alcoholic fatty liver disease. International Journal of Clinical and Experimental Medicine. 7(11), 4191-8.
15 Feldstein et al, 2013. Serum Cytokeratin-18 Fragment Levels Are usful Biomarker for Nonacoholic Steatohepatis in Children. American Journal of Gastroenteroly. 108(9), 1526-3.
16 Fitzpatrick et al, 2010. Serum levels of CK18 M30 and leptin are useful predictors of steatohepatitis and fibrosis in paediatric NAFLD. Journal of Pediatric Gastroenteroly and Nutrition. 51(4), 500-6.

VLVbio

Hästholmsvägen 32, 131 30 Nacka, Sweden
Telephone: +46 8 122 053 00
e-mail: info@vlvbio.com • www.vlvbio.com