# M30 Apoptosense® ELISA

in detecting NAFLD

*The dead cells still count!*

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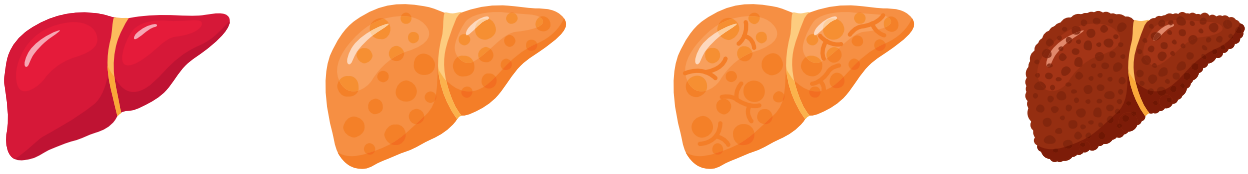
# NAFLD and NASH

Non-alcoholic fatty liver disease (NAFLD), a condition where fat accumulates in the liver without the excess consumption of alcohol, has become the most common cause of liver disease in the Western world, and is quickly rising to become the primary cause of liver transplants. Due to the rise in obesity and diabetes, NAFLD is estimated to affect 24 % of the global population, with a high prevalence on all continents. This increase is most likely related to the so-called Western lifestyle; fast food, lifestyle changes, and reduced physical activity. NAFLD starts out as steatosis which is an accumulation of fat in the liver and may further progress to non-alcoholic steatohepatitis (NASH), a more serious form of NAFLD, where the liver has become inflamed. NASH is a potentially fatal condition that affects 12 % of the global adult population and can further develop into creation of fibrotic tissue in the liver, with possible cirrhosis as a result.

## NAFLD process

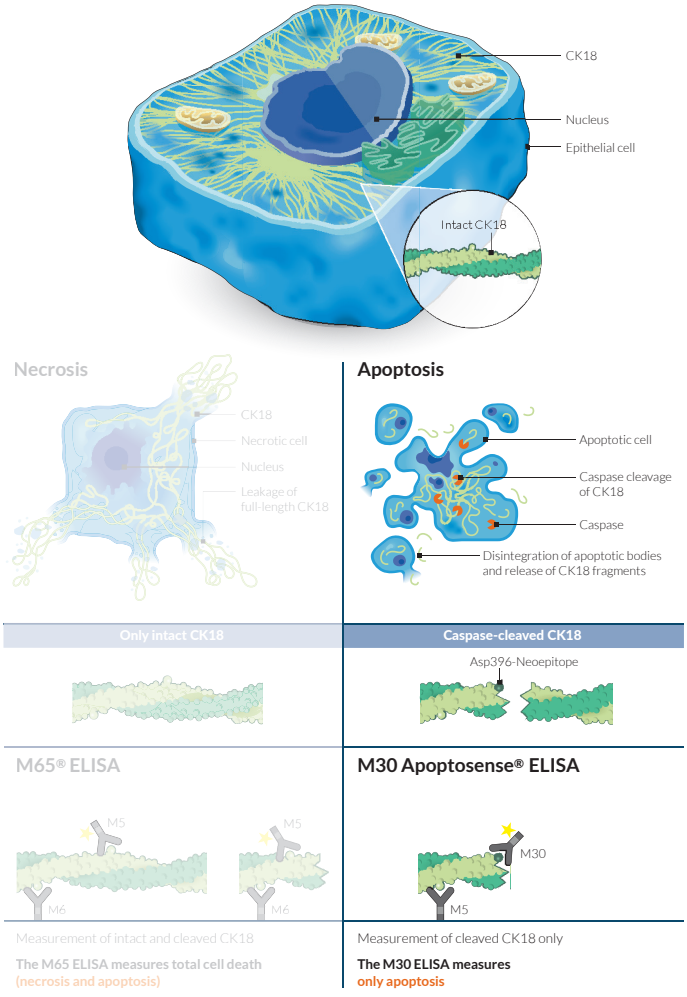


healthy liver | hepatic steatosis | steatohepatitis (NASH) | liver cirrhosis

## The role of keratin 18 in NAFLD

Keratin 18 (K18) is an intermediate filament protein forming part of the cytoskeletal structure expressed in epithelial cells. Hepatocytes, a form of epithelial cell, are the main tissue cells found in the liver. When simple steatosis in NAFLD is accompanied by inflammation of hepatocytes, the disease is described as NASH. This prominent characteristic of the disease is mainly caused by hepatocyte cell death due to apoptosis. Early on in the apoptosis of hepatocytes, caspases are activated which cleave the K18 protein, and the resulting fragments are subsequently released into the blood. These caspase cleaved K18 (ccK18) fragments can be efficiently quantified by the unique M30 Apoptosense® ELISA.

Standard biomarkers of liver injury can be valuable tools to indicate a problem with the liver, but are non-specific and may perform poorly in the early detecting of NAFLD and NASH. Therefore, it is essential to complement the use of standard biomarkers in order to increase the accuracy for detecting and stratifying NAFLD and NASH patients at an early stage, before advancements into end stage liver disease. During early stages of NAFLD, the patient is often asymptomatic, and lifestyle intervention may help reverse the disease. However, it is much more difficult to reverse late stage liver disease where fibrosis has developed in large parts of the liver. Hence, a non-invasive early stage detection of NAFLD is important.



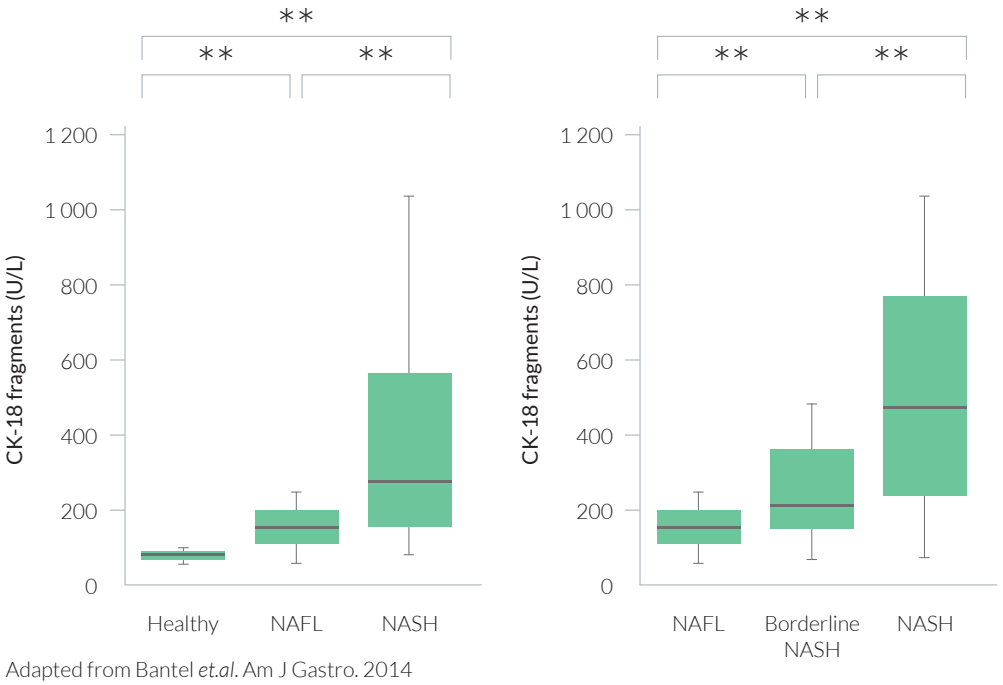
The detection of NAFLD and NASH is today largely dependent on liver biopsy which is invasive, extensive, and expensive. Since apoptosis is a well studied mechanism for NAFLD progression, the availability of a serum biomarker for hepatic apoptosis may widen the availability of the detection of NAFLD and NASH. A non-invasive biomarker that detects the disease at an early stage and is also time- and cost efficient, while reducing patient discomfort and hospitalization, is of importance.

## The M30 Apoptosense® ELISA:

- ✓ Is a sensitive indicator of NAFLD, including NASH
- ✓ Has the potential to reduce the number of liver biopsies
- ✓ Can aid in NAFLD staging


### M30® levels in patients with NAFLD:

The M30 Apoptosense® ELISA allows for the significant discrimination between NASH and NAFLD or healthy individuals, while also distinguishing between NAFL and healthy controls.




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

**Example 1:**
Bantel *et al*[1]. studied ccK18 fragments for the detection and staging of NAFLD in 74 subjects with NASH, 23 with Non-Alcoholic Fatty Liver (NAFL) and 23 healthy controls.

**Findings:**
Statistically significant differences in serum M30® levels were found between the three groups.

**The authors state:**
*"Thus, we provide the important information that the improved M30-ELISA represents a robust and sensitive method for detection of relevant steatosis and for staging of NAFLD."*

**Example 2:**
Habib *et al*[2]. studied serum ccK18 fragments, with the M30 Apoptosense® ELISA, in 46 subjects with NASH, 54 subjects with Non-NASH (i.e. borderline NASH, simple steatosis or normal liver histology on biopsy), and 30 healthy controls. The severity of NASH was graded with the NAFLD Activity Score (NAS).

**Findings:**
Serum levels of M30® differed significantly between the three groups; healthy controls, those with Non-NASH, and those with NASH.

**The authors state:**
*"Serum CK-18 levels were significantly correlated to the disease severity as measured by liver biopsy (degree of steatosis, fibrosis, lobular inflammation, and ballooning) in NASH patients. Serum CK-18 could be used as a noninvasive diagnostic serum marker for patients of NAFLD and NASH."*

# M30 Apoptosense® ELISA in diagnosing fibrotic NASH

The pathology of NASH exhibits several parameters; metabolic disturbances, inflammation, fat deposition, cell death and, later fibrosis. Several studies suggest that NASH with fibrosis is an important prognostic factor for predicting the risks of mortality and liver related morbidity in NAFLD patients. Hence, detecting fibrotic NASH patients has become important in order to identify those patients most likely to benefit from treatment intervention. Therefore, optimal biomarker panels for the screening and detection of fibrotic NASH should contain a combination of biomarkers. The common criteria used for identifying fibrotic NASH are NAS≥4 and fibrosis stage F≥2. M30 Apoptosense® ELISA is a sensitive and specific biomarker for hepatocyte apoptosis and can greatly increase the accuracy of biomarker panels for fibrotic NASH. In particular, including the M30® assay in panels can reduce the number of subjects who may require further investigation with liver imaging technologies and eventually biopsies.

**Example 1:**
Liebig *et al*[3]. studied whether the M30 Apoptosense® ELISA might identify NAFLD patients who are at risk of NASH and fibrosis despite a low NAFLD Fibrosis Score (NFS) or Transient Elastograhy (TE) values. Serum ccK18 levels were assessed in combination with NFS and/or TE in exploration and validation cohorts of patients with biopsy-proven NAFLD.

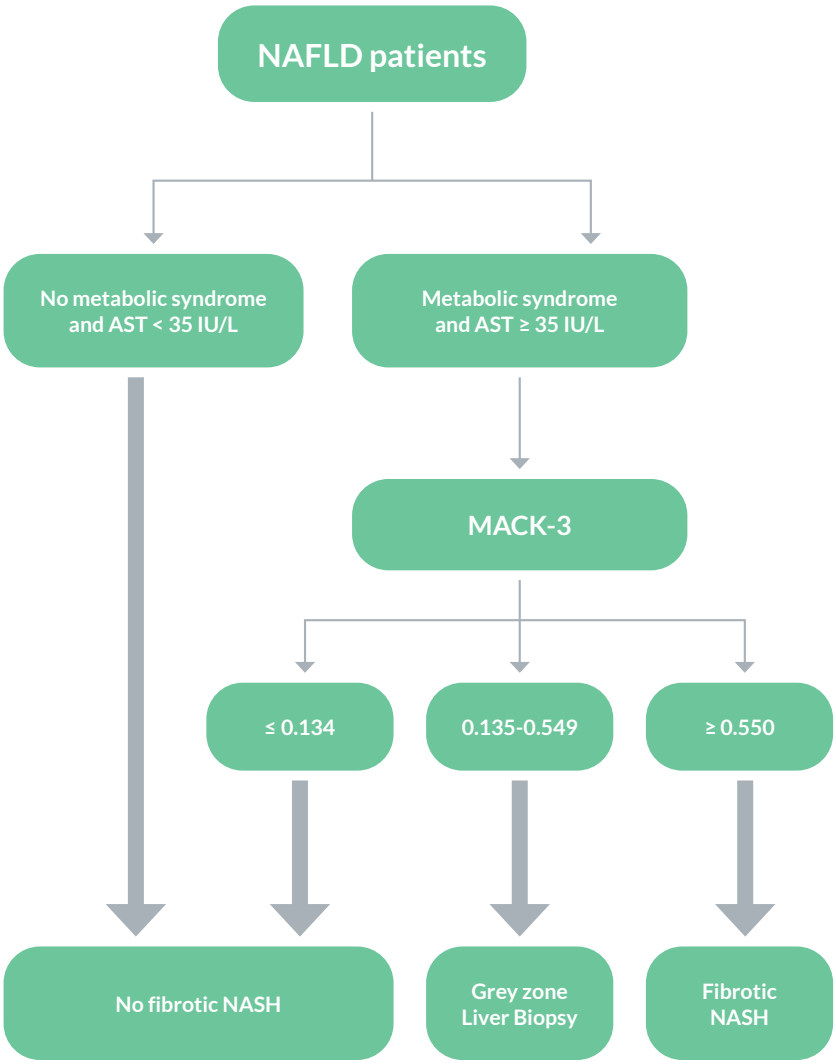
**Findings:**
Over 60 % of patients with low NFS had increased serum ccK18 levels, and of these 70 % had NASH, mostly with histological fibrosis. In contrast, most subjects with low NFS scores and non-elevated ccK18 levels did not show NASH. The majority of subjects with intermediate NFS scores had elevated ccK18 levels and, of these, 90 % showed fibrosis. Similar results were obtained when M30 Apoptosense® ELISA results were combined with TE.

**The authors state:**
*"These results demonstrate that the combination of the M30® marker with NFS or TE enables a more reliable and accurate identification of patients with an increased risk of progressed NAFLD."*

The M30 Apoptosense® ELISA has not been approved by the U.S. Food and Drug Administration.

# MACK-3 in Clinical Practice

Patient flowchart implementing MACK-3 into clinical practice.




Adapted from Boursier *et. al.* Aliment Pharmacol Ther. 2018

**Example 2:**
To evaluate the diagnostic accuracy of blood fibrosis tests, Boursier *et al*[4]. compared NFS, BARD score and Fibrosis-4 index (FIB-4) with biomarkers of metabolic syndrome (MetS), insulin resistance, liver inflammation and ccK18 for the diagnosis of fibrotic NASH in a study on 846 subjects with biopsy proven NAFLD. Data for these biomarkers were then used to develop a new biomarker panel aimed at improving the diagnostic accuracy of NAFLD.

**Findings:**
The blood fibrosis tests; BARD score, NFS and FIB-4, had poor accuracy for fibrotic NASH. Fibrotic NASH was independently predicted by AST, homeostatic model assessment (HoMa) and ccK18 using the M30 Apoptosense® ELISA, and these were combined in a new blood test MACK-3. These biomarkers are associated with liver inflammation (AST), insulin resistance (HoMa), and apoptosis (ccK18), supporting the patho-physiological relevance of the MACK-3 panel.

MACK-3 had a significantly higher diagnostic accuracy than the other blood fibrosis tests.

**The authors state:**
*"The new blood test MACK-3 accurately diagnoses fibrotic NASH. This new test will facilitate patient screening and inclusion in NAFLD therapeutic trials and will enable the identification of patients who will benefit from the treatments once approved."*

**Example 3:**
The MACK-3 algorithm was further independently validated by Chuah *et al*[5], who studied 196 biopsy proven NAFLD patients. The accuracy of MACK-3 to predict the presence or absence of fibrotic NASH was evaluated and compared with the NFS, FIB-4 and BARD scores.

**Findings:**
MACK-3 was good for the diagnosis of fibrotic NASH and was also good for diagnosis of active NASH*, performing better than the other blood fibrosis tests.

NFS was evaluated on patients with intermediate MACK-3 results. Patients with high NFS values were then biopsied and this resulted in a diagnostic accuracy of 99.5 % for detecting fibrotic NASH.

**The authors state:**
*"MACK-3 is promising as a non-invasive test for active NASH and fibrotic NASH and may be useful to identify patients who need more aggressive intervention."*

*Active NASH was defined as NASH with NAS≥4, while fibrotic NASH was defined as presence of active NASH and F≥2


| VLVbio™ Product Line | |
|---|---|
| **ELISA Products** | **Prod. No** |
| M30 Apoptosense® ELISA | 10011 |
| M30 CytoDeath™ ELISA | 10900 |
| M65® ELISA | 10020 |
| M65 EpiDeath® ELISA | 10040 |
| M65 EpiRat™ ELISA | 10060 |

## How to order

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**References:**

[1] Bantel *et al*, 2014. Robust Detection of Liver Steatosis and Staging of NAFLD by an Improved ELISA for Serum Cytokeratin-18 Fragments. American Journal of Gastroenterology. 109(1), 140-1.

[2] Habib *et al*, 2019 Value of Cytokeratin-18 as a non-invasive diagnostic biomarker of nonalcoholic steatohepatitis (NASH). Medical Journal of Viral Hepatitis. 4(2), 65-74.

[3] Liebig *et al*, 2019. Multicenter Validation Study of a Diagnostic Algorithm to Detect NASH and Fibrosis in NAFLD Patients with Low NAFLD Fibrosis Score or Liver Stiffness. Clinical and Translational Gastroenterology. 10(8).

[4] Boursier *et al*, 2018. Screening for therapeutic trials and treatment indication in clinical practice: MACK-3, a new blood test for the diagnosis of fibrotic NASH. Alimentery Pharmacology & Therapeutics. 47(10), 1387-96.

[5] Chuah *et al*, 2019. MACK-3 (Combination of hoMa, Ast and CK18): A promising novel biomarker for fibrotic non-alcoholic steatohepatitis. Liver International. 39(7), 1315-24.

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