M30 Apoptosense ELISA
A biomarker assay for detection and screening of NASH
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

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 simple 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
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.
Chen et al. conclude, by a meta-analysis of 10 studies including 838 patients, that K18 fragments has a clinically meaningful 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.

"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." Dvorak et al. conclude that K18 fragments have shown the most consistent results for differentiating NASH from steatosis.

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.

**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.

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.
**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 assessed 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.

**Apoptosis**
- M30 level: caspase-cleaved K18 (ccK18)
- M65 level: total K18

High levels of caspase-cleaved K18 (ccK18) compared to total K18 (high M30:M65 ratio)

**Necrosis**
- M30 level: caspase-cleaved K18 (ccK18)
- M65 level: total K18

Low levels of caspase-cleaved K18 (ccK18) compared to total K18 (high M30:M65 ratio)

---

**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

<table>
<thead>
<tr>
<th>ELISA Products</th>
<th>Prod. No</th>
<th>Apoptosis</th>
<th>Total cell death</th>
</tr>
</thead>
<tbody>
<tr>
<td>M30 Apoptosense® ELISA</td>
<td>10011</td>
<td>✔</td>
<td>–</td>
</tr>
<tr>
<td>M65® ELISA</td>
<td>10020</td>
<td>–</td>
<td>✔</td>
</tr>
</tbody>
</table>

Other PEVIVA Line Products

<table>
<thead>
<tr>
<th>ELISA Products</th>
<th>Prod. No</th>
</tr>
</thead>
<tbody>
<tr>
<td>M30 CytoDeath™ ELISA</td>
<td>10900</td>
</tr>
<tr>
<td>M65® ELISA</td>
<td>10040</td>
</tr>
<tr>
<td>Monoclonal Antibody Products</td>
<td>Prod. No</td>
</tr>
<tr>
<td>M5 Keratin 18 mAb</td>
<td>10600</td>
</tr>
<tr>
<td>M6 Keratin 18 mAb</td>
<td>10650</td>
</tr>
<tr>
<td>M30 CytoDEATH™ mAb (unlabelled)</td>
<td>10700</td>
</tr>
<tr>
<td>M30 CytoDEATH™ mAb Biotin</td>
<td>10750</td>
</tr>
<tr>
<td>M30 CytoDEATH™ mAb Fluorescein</td>
<td>10800</td>
</tr>
<tr>
<td>M30 CytoDEATH™ mAb Orange</td>
<td>10830</td>
</tr>
</tbody>
</table>

REFERENCES