3D InSight™ Human Liver NASH Model

The InSphero 3D InSight™ Human Liver NASH Model is a human liver microtissue designed for the study of mechanisms of non-alcoholic steatohepatitis (NASH) induction (Figure 1) and in vitro screening of anti-NASH drug efficacy. The model includes all the critical liver cells and inducers needed to recapitulate the inflamed fatty liver disease state and serves as a powerful model for NASH drug discovery and development.

- Rely on a physiologically relevant model composed of primary human hepatocytes (PHHs), hepatic stellate cells (HSCs), Kupffer cells, and liver endothelial cells (LECs)
- Expand your assay window by leveraging a pre-qualified model that exhibits pro-inflammatory marker induction reflecting the in vivo disease state: MCP-1/CCL2, TNF-α, and MIP-1α/CCL3
- Perform reproducible, throughput-compatible anti-fibrotic drug screening using a standardized, assay-ready 96-well model system, amenable to a host of certified application endpoints

### Certified Applications

<table>
<thead>
<tr>
<th>Designed for assessment of:</th>
<th>Options:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficacy screening of anti-NASH and anti-fibrosis drugs</td>
<td>✅</td>
</tr>
<tr>
<td>Anti-NASH drug mechanism of action</td>
<td>✅</td>
</tr>
<tr>
<td>Combined efficacy and toxicity testing of anti-NASH drugs</td>
<td>✅</td>
</tr>
<tr>
<td>Mechanism of disease progression</td>
<td>✅</td>
</tr>
<tr>
<td>Contribution of cell types to disease progression</td>
<td>✅</td>
</tr>
<tr>
<td>Testing pro-NASH potential of drugs</td>
<td>✅</td>
</tr>
</tbody>
</table>

**Figure 1: Disease Model Concept.** The 3D InSight™ Human Liver NASH Model enables the study of liver NASH induction and inhibition of disease progression. The screening-compatible Akura™ 3D microtissue format provides maximal endpoint compatibility while optimizing efficiency of microtissues handling processes.
Mechanistically relevant anti-NASH drug screening

Liver NASH Model Characterization Data

Induction of NASH not only requires the presence of hepatocytes, in which large lipid vacuoles accumulate during steatosis, but also HSCs, which are activated by pro-inflammatory and pro-fibrotic stimuli. In addition, Kupffer cells (specialized macrophages located in the liver) and LECs influence the progression of inflammation and are thus required for a physiologically, mechanistically relevant in vitro model system. The 3D InSight™ Human Liver NASH model includes all these relevant primary liver cell types. Upon treatment with FFA, LPS and sugars, the model reflects increased lipid accumulation within hepatocytes, secretion of pro-inflammatory biomarkers, and deposition of fibrillar collagens. Treatment with Elafibranor, a PPARα/δ agonist under development by GenFit, resulted in decreased secretion of pro-inflammatory markers MCP-1/CCL2, TNF-α, and MIP-1α/CCL3 (Figure 2). Concomitant treatment of FFA and LPS with an ALK5 inhibitor blocked HSC activation and collagen deposition (Figure 3), demonstrating suitability of the model system for testing anti-NASH compound efficacy.

Figure 2: Secretion of inflammatory markers decrease with Elafibranor treatment. Secretion of inflammatory markers increase upon induction of NASH with FFA, LPS, and sugars. Treatment with Elafibranor decreased secretion of these biomarkers. *P<0.05; **p<0.005 comparison between FFA+LPS and FFA+LPS/Elafibranor-treated samples.

Figure 3: Histological characterization after NASH disease induction with inhibition. Induction of the liver fibrosis associated with NASH is confirmed by elevated expression of stellate cell activation marker α-SMA, ECM markers Col I, Col III, and Col IV. Presence of fibrillar collagens, associated with scarring of the liver, are shown with Sirius Red staining and polarized light. Simultaneous treatment with FFA, LPS, sugars, and ALK5 inhibitor rescues the liver phenotype and halts progression to liver fibrosis.

3D InSight™ Human Liver NASH Model

Model Specifications
- **Microtissue**
  3D InSight™ Human Liver Co-culture (PHHs, Kupffer cells, HSCs, and LECs)
- **Media**
  3D InSight™ Human Liver Advanced GlucoTox Medium
- **Format**
  Akura™ 96 plate 96 microtissues, 1 tissue/plate

Lipid-loaded NASH disease model

Related Models and Platforms

3D InSight™ Liver Disease Discovery Platform
- 3D InSight™ Human Liver Fibrosis Model
- 3D InSight™ Human Liver Steatosis Model

3D InSight™ Diabetes Discovery Platform
- 3D InSight™ Diabetes Type II Model
- 3D InSight™ Diabetes Type I Model

3D InSight™ Liver Toxicology Platform