

# CELLBLOKS® NANOSTACKS™

Build *in vivo*-relevant organotypic structures

## Background

- Commonly used *in vitro* models are typically based on monocultures of single cell types
- There is a growing need for *in vitro* models based on the co-culture of 3+ cell types, to model the biological complexity observed *in vivo*
- **CELLBLOKS® Stacks™** provide a user-friendly solution to assemble miniaturised *in vitro* models including up to 4 different cell types



### Transparent

Compatible with fluorescent and standard light microscopy



### 3D

Assemble up to 4 Stacks™ and build multi-layered *in vitro* models



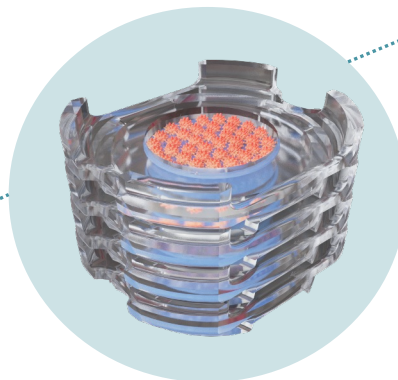
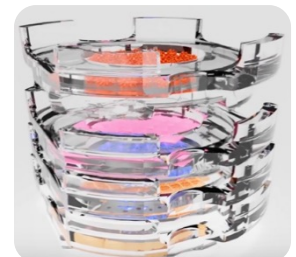
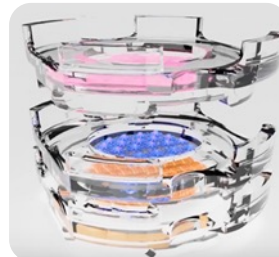
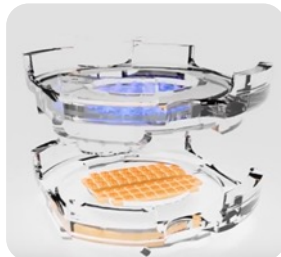
### Industry-compliant

Stacks™ can fit into a SBS-standard 24 well-plate

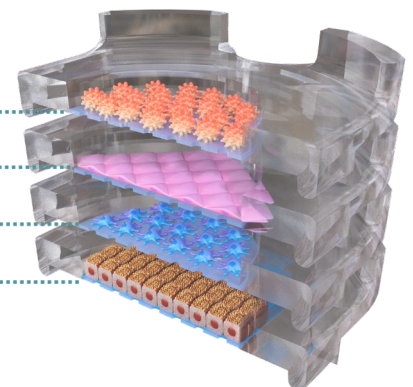


### Assay-friendly

Compatible with plate readers and standard laboratory equipment



Permeable, optically transparent membranes



# Data

## Application: enhanced hepatic function on 3D liver model

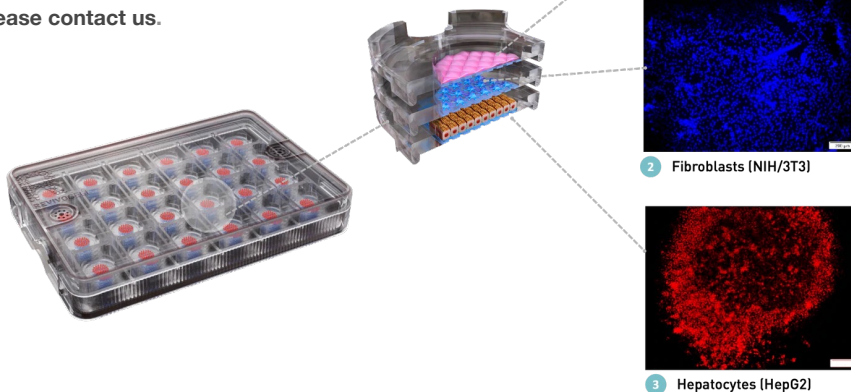
Application: Stacks™ can be used for the development of a 3D liver model displaying improved hepatic function compared to 2D monocultures.

For this application, endothelial cells (HUVEC), fibroblasts (NIH/3T3), and hepatocytes (HepG2) were seeded on 3 different Stacks™.

Additionally, the use of primary human hepatocytes was tested.

The HUVEC-NIH/3T3-HepG2 Stacks™-based 3D liver model is available on demand.

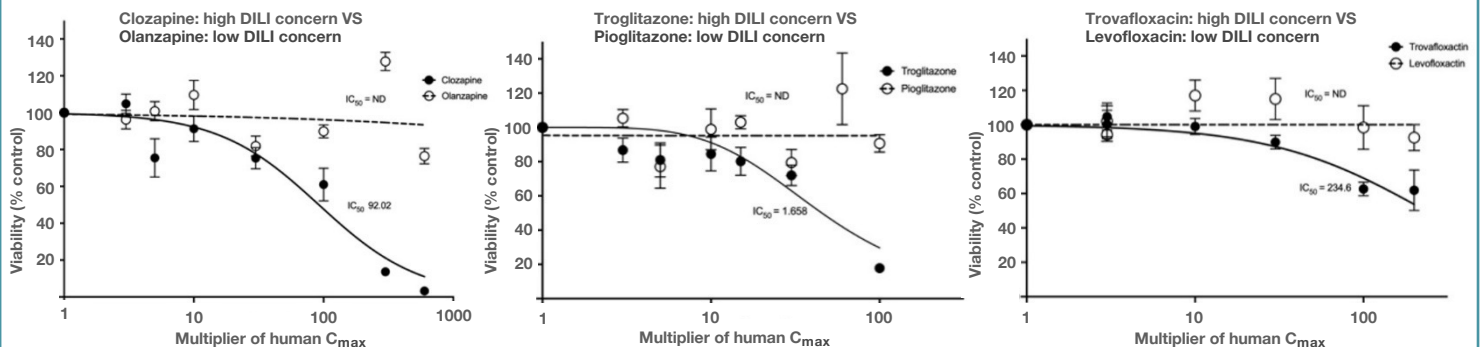
For the development of other bespoke models, please contact us.



**Figure 1. Live imaging of cells in Stacks™-based 3D liver model.**

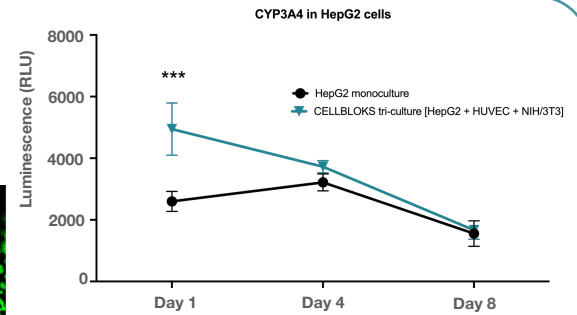
Cells were imaged live on day 3 in stacked layers. HUVEC: CellTracker™ Green CMFDA (Thermo Fisher Scientific), HepG2: CellTracker™ Red CMTPIX (Thermo Fisher Scientific), NIH/3T3: Hoechst 33342 (ThermoFisher Scientific). **Cells can be easily imaged through the layers of Stacks™ using a standard inverted microscope.**

Magnification: 10 X. Scale bars: 200 µm.



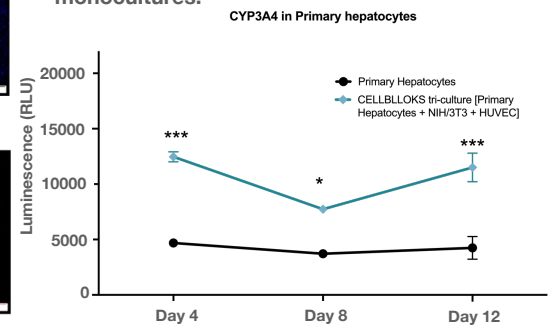
**Figure 4. Hepatotoxicity evaluation (24 h exposure) in Stacks™-based 3D liver model.**

Hepatotoxic drug-induced toxicity in HepG2 cells can be detected at concentrations < 10 µM over human plasma C<sub>max</sub> levels. The model correctly differentiated the hepatotoxic effects of compounds at high DILI concern (Clozapine, Troglitazone, Trovafloxacin) as opposed to their non-toxic drug analogues (respectively Olanzapine, Pioglitazone, Levofloxacin) at clinically relevant concentrations, therefore demonstrating a **robust DILI prediction capability**.



**Figure 2. Metabolism in HepG2 hepatocytes.**

CYP3A4 activity levels in monocultures of HepG2 on a single Stack™ VS tri-culture with HUVEC and NIH-3T3 on 3 Stacks™. **In tri-cultures, HepG2 CYP3A4 activity increased in the first 24 h compared to monocultures.**



**Figure 3. Metabolism in human upcyte® human primary hepatocytes.**

CYP3A4 activity levels in monocultures of human primary hepatocytes on a single Stack™ VS tri-culture with HUVEC and NIH/3T3 on 3 Stacks™. **In tri-cultures, CYP3A4 activity of primary hepatocytes is enhanced compared to monocultures.**

## Product specifications

|                     |   |
|---------------------|---|
| Base plate          | SBS-standard 24 well-plate  |
| Working well volume | 1.2 mL per well   |
| Stacks™ properties  | Body material: optically transparent medical grade polycarbonate<br>Cell growth surface material: transparent polyester porous membrane (0.4 µm pore size, 2 x 10 <sup>6</sup> pores/cm <sup>2</sup> )<br>Cell growth area: 7.69 mm <sup>2</sup><br>Stacks™ height: 3.4 mm for single Stack™, 7.2 mm for 3 Stacks™ assembled together |