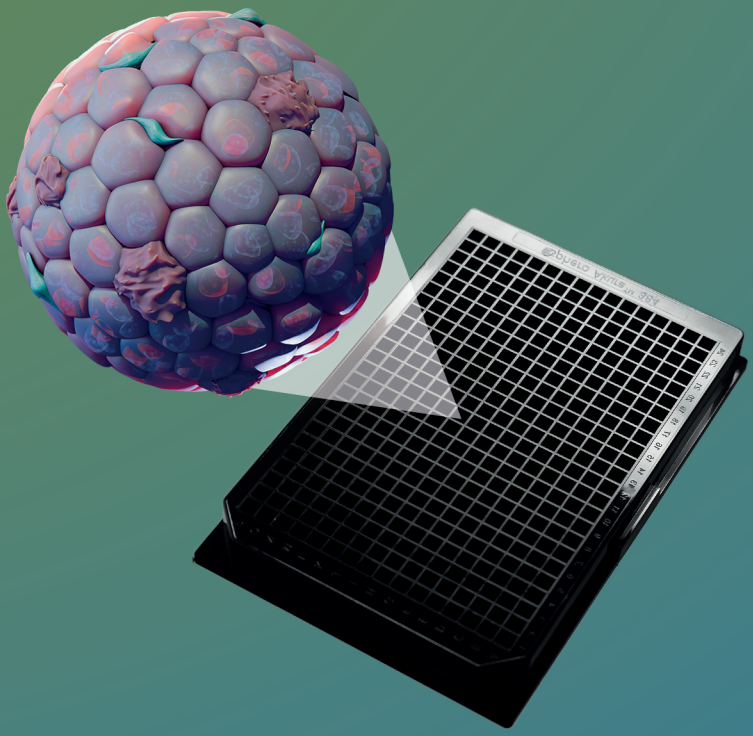
insphero

The Model of Excellence™

3D InSight™ *in vitro* Liver Toxicology

Predictive Toxicology Services



Screen and identify toxicity hazards with physiologically-relevant 3D InSight™ Liver Microtissues

Industry-ready 3D *in vitro* DILI Testing

3D InSight™ Liver Microtissues - The gold standard in Human-Relevant 3D *in vitro* models

300,000	35,000	55 +	10,000+
3D Human Liver Microtissues produced in 2024	3D Species-Specific Liver Microtissues produced in 2024	Drug Developers adopted 3D InSight™ Liver Microtissues in 2024	Drug Candidates Tested

Physiological 3D liver models for confident hepatotoxicity screening

Screen for hepatotoxicity with 3D InSight™ Liver Microtissues, physiological co-cultures of primary hepatocytes and non-parenchymal liver cells from human or preclinical species.

Qualified 4-week long viability

Up to four weeks of viability and hepatic function enable low-concentration, long-term exposure studies, that allow for the detection of chronic effects.

Faster go/no-go decisions with standardized hepatotoxicity screening

Accelerate your programs with standardized, high-throughput hepatotoxicity screening, available weekly for human models and monthly for animal models. Within 10 working days, receive a comprehensive analysis report to support timely decisions and keep your projects moving forward.

One liver model, deeper insight

Complement your cytotoxicity "DILI" assay with one or more of the available and optional biomarkers in order to explore the effect of different compounds in depth.

High-density microplates for accuracy and scale

96- and 384-well formats provide robust datasets at scale, with 7 concentrations tested in 4 replicates per assay for accurate, reproducible measurements.

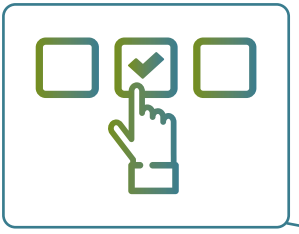
Extensively published and broadly used 3D liver models

Build confidence in your data with 3D liver models, featured in scientific literature by leading pharmaceutical companies and regulatory bodies (see publication lists on pages 14 &15). Infer the *in vivo* effect of your compounds by comparing their *in vitro* results with our published datasets of hundreds of marketed drugs screened in 3D liver microtissues.

- 7-day DILI assay; Akura™ 384 Spheroid Microplate; Fäs et al., *Toxicological Sciences*, 2025
- 14-day DILI assay; Akura™ 96 Spheroid Microplate; Proctor et al., *Archives of Toxicology*, 2017

How to get started with our DILI Testing service

Getting started with our DILI Testing service is simple. Choose the solution that best fits your program, ship your compounds, and let our experts handle the testing. You'll receive high-quality, actionable data in a comprehensive report to support confident decision-making.

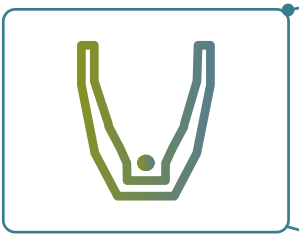


Select the right assay for your program

Choose DILI testing aligned with your preclinical strategy, from early hazard identification to advanced multi-parametric readouts.

Simplified compound logistics

Ship with ease. We coordinate compound logistics and 3D liver microtissue preparation in parallel to keep your study timelines on track.



Expert-driven *in vitro* DILI testing

Our scientific team runs your study using optimized dosing, validated workflows, and robust readouts for high-confidence results.

Comprehensive, decision-ready reporting

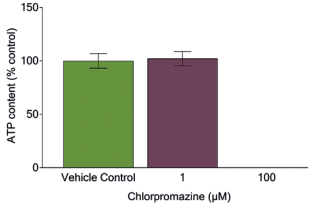
Receive a publication-grade report with actionable insights for internal reviews, regulatory submissions, and portfolio decisions within 10 business days of experimental completion.

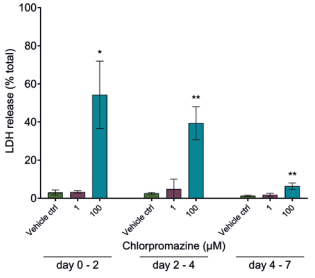
DILI Flag

Screen compound libraries for cytotoxicity using gold-standard, physiologically relevant liver models.

Context of use: Exclude cytotoxic chemical series and compounds at the lead identification phase

Species	Human
Plate Format	Akura™ 384 Spheroid Microplate
Treatment Duration	7-day, compound applications: Day 0, 2 & 5 14-day, compound applications: Day 0, 5 & 9
Test Groups	Conditions: 2 concentrations in 4 replicates Positive control: specific to drug modality Vehicle control: Yes, 0.5% (v/v) DMSO or 5% (v/v) PBS
Measurement	DILI Flag: ATP (endpoint)
Quality Control Criteria	100 uM Chlorpromazine: ▪ Complete loss of cellular ATP; 1 uM Chlorpromazine: no statistically significant cellular ATP reduction or LDH leakage
Drug Candidate Modality	Including but not limited to small molecules, oligonucleotides, AAVs, biologics
Data Delivery	Tabular format (inc. raw data and plate map) [Optional]: Basic written report
Note	Minimal number of compounds per submission: 8





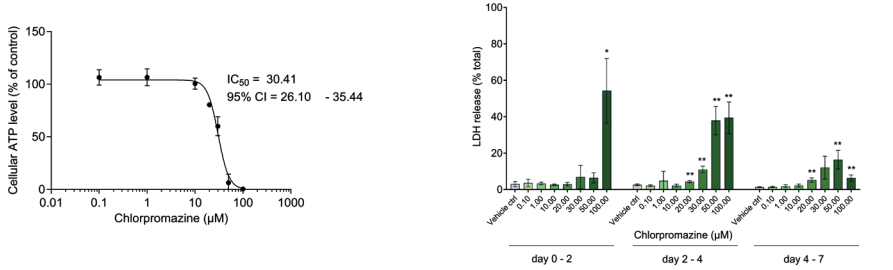
Exemplary data showing cytotoxicity induced by chlorpromazine in human liver microtissues in 7-day DILI Flag Plus assay

DILI / DILI Plus

Accurate ATP IC_{50} determination, customizable with InSphero's full suite of biomarker assays

Context-of-Use: 1/ Precisely Rank order drug candidates for cytotoxicity at the lead optimization stage; 2/ Before First-in-Human trials: Verify margin of safety (PMID: 39397666)

Species	Human, and Species-Specific Liver Microtissues (Cynomolgus Monkey, Beagle Dog, Sprague-Dawley Rat, C57BL/6 mouse)
Plate Format	Akura™ 96 Spheroid Microplate ; Akura™ 384 Spheroid Microplate Note: studies in 384 plates are available only in human
Treatment Duration	7-day, compound applications: Day 0, 2 & 5 14-day, Compound applications: Day 0, 5 & 9 21-day, Compound applications: Day 0, 5, 9, 14 & 19 28-day, Compound applications: Day 0, 5, 9, 14, 19 & 23 Note: rat studies available only up to 7-days
Test Groups	Conditions: 7 concentrations in 4 replicates Positive control: Yes, Drug candidate modality & species-specific Vehicle control: Yes, 0.5% (v/v) DMSO or 5% (v/v) PBS
Measurement	DILI: ATP (endpoint) DILI Plus: ATP (endpoint) and LDH activity leakage (kinetic, at each medium collection)
Quality Control Criteria	Timepoint-dependent and according to historical positive control values
Drug Candidate Modality	Including but not limited to small molecules, oligonucleotides, AAVs, biologics
Data Delivery	Tabular format (inc. raw data and plate map) [Optional]: Basic written report [Optional]: Regulatory-friendly written report



Exemplary data showing cytotoxicity induced by chlorpromazine in human liver microtissues in 7-day DILI Plus assay in Akura™ 384 Spheroid Microplate (left: ATP; right LDH leakage).

Unlock DILI Results

Turn margin of safety into actionable insight through rigorous peer-reviewed *in vitro*-to-*in vivo* comparative studies

Human DILI Assay

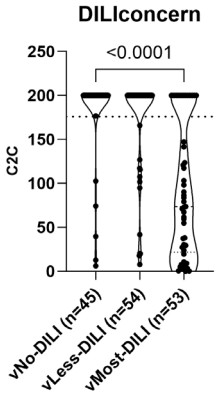
ALL MODALITIES

Safety ranking of Lead Optimization selection

SMALL MOLECULES

Evaluate safe exposure for orally administered small molecules

- **7-day DILI assay in 384-well plate**
  $IC50_{ATP}$/ Cmax < 176 distinguishes safe from hepatotoxic compounds (Fäs et al., Toxicol. Sci., 2025).
- **14-day DILI Assay in 96-well plate**
  $IC50_{ATP}$<100 µM distinguishes safe from hepatotoxic compounds (Proctor et al., Arch. Toxicol., 2017).

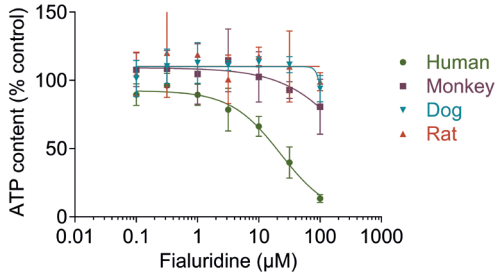


Adapted from Fäs et al., Toxicol. Sci., 2025. IC50ATP/ Cmax of 152 FDA-approved orally administered drugs is significantly different between no-DILI and Most-DILI concern drugs

Species-Specific* DILI Assay

ALL MODALITIES

Before or after *in vivo* preclinical studies: De-risking species-specific hepatotoxicity by comparing $IC50_{ATP}$ from preclinical species and human liver microtissues



7-day dose–response curve of 3D InSight™ Species-Specific Liver Microtissues* treated with fialuridine. ATP measurements (mean $IC50_{ATP}$ ± 95% CI) reveal human-specific cytotoxicity, aligning with *in vivo* findings.

*3D InSight™ Species-Specific Liver Microtissues (Cynomolgus Monkey, Beagle Dog, Sprague-Dawley Rat, C57BL/6 mouse)

Multiplexing of DILI Services

A comprehensive range of biomarker that can be multiplexed with customized services and standardized DILI/DILIplus assays.

If requested, during DILI or DILI Plus assays the supernatant from each medium exchange and the final time point can be stored for 3 months at -80°C. The table below lists the available biomarkers and their volume requirements, helping determine which assays can be combined, as approximately 50 µL of supernatant is preserved for each time point.

Soluble Biomarkers Quantifiable in Supernatants

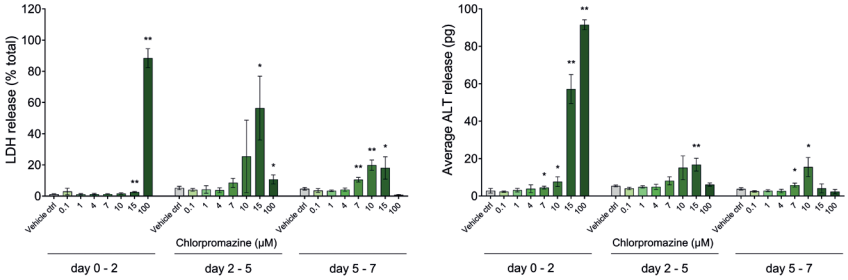
Adverse Event	Clinical Marker	*In Vitro* Biomarker	Species	Volume Required
Necrosis	ALT	LDH Activity	All	5 µl
Necrosis	ALT	ALT	Human; Rat	96w: 25 µl 384w: 12.5 µl
Apoptosis	ccCK 18	ccCK 18	Human	20 µl
Apoptosis	ccCK 18	Caspase 3/7 Activity	All	20 µl
Hepatocyte Function	Albumin	Albumin	All	All except rat: 5 µl; Rat: 10 µl
Mitochondrial Function	Lactate	Lactate	All	2.5 µl
Inflammation	IL-6	Luminex	Please Inquire	96w: 25 µl 384w: 12.5 µl

***3D InSight™ Species-Specific Liver Microtissues (Cynomolgus Monkey, Beagle Dog, Sprague-Dawley Rat, C57BL/6 mouse)**

Necrosis and Apoptosis Markers

Leakage Markers: LDH Assay and ALT ELISA

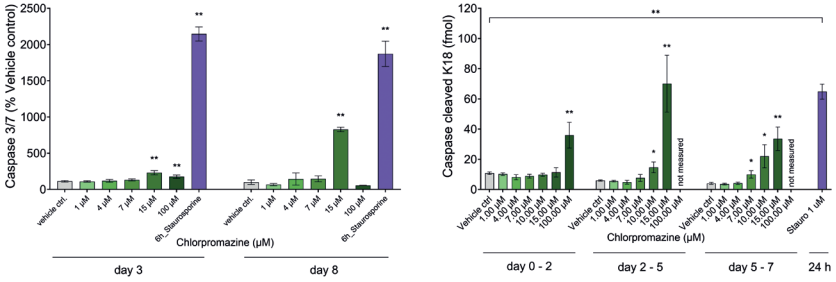
Measurement of leakage markers provides a kinetic overview of cytotoxicity and necrosis induced by the test item. The LDH Assay is available in human and species-specific microtissues*. ALT ELISA is available in Human and Sprague-Dawley Rat Liver Microtissues. Multiplexing is possible with all other biochemical assays.



Exemplary data showing loss of membrane integrity induced by Chlorpromazine in human liver microtissues (left: LDH; right: ALT).

Apoptosis Markers: Caspase 3/7 Activity Assay and caspase-cleaved keratin 18 (ccK18) ELISA

Measuring apoptosis markers provides a kinetic overview of the onset of apopotosis induced by the test item. Caspase 3/7 Activity Assay is available in human and species-specific microtissues* and ccK18 ELISA is available in Human Liver Microtissues. Multiplexing is possible with all other biochemical assays.



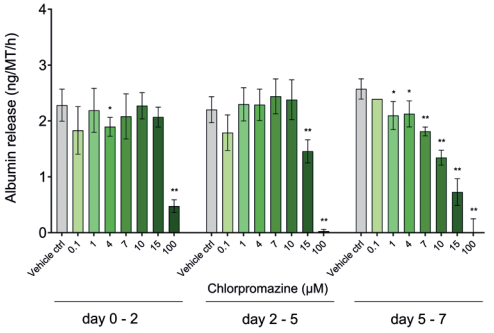
Exemplary data showing onset of apoptosis induced by Chlorpromazine in human liver microtissues in 7-day treatment (left: Caspase 3/7 activity; right: ccK18 ELISA)

***3D InSight™ Species-Specific Liver Microtissues (Cynomolgus Monkey, Beagle Dog, Sprague-Dawley Rat, C57BL/6 mouse)**

Other Liver Functionality Markers

Hepatocyte Functionality: Albumin ELISA

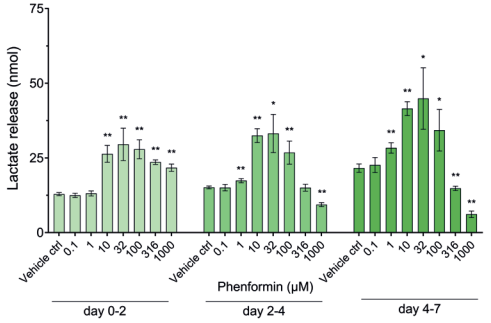
Hepatocytes in liver microtissues maintain stable albumin synthesis for up to 28 days. A decrease in albumin production after treatment indicates potential hepatotoxicity. Albumin ELISA is available in human and species-specific microtissues.



Exemplary data showing decrease of albumin levels induced by chlorpromazine human liver microtissues

Mitochondrial Functionality Assay: Lactate Release Assay

Lactate Release Assay evaluates how a substance modulates mitochondrial function. For example, if a drug inhibitis mitochondrial oxidative phosphorylation this will be detected as an increase in lactate. Lactate Release Assay is available in human and species-specific microtissues. Multiplexing is possible with all other biochemical assays.

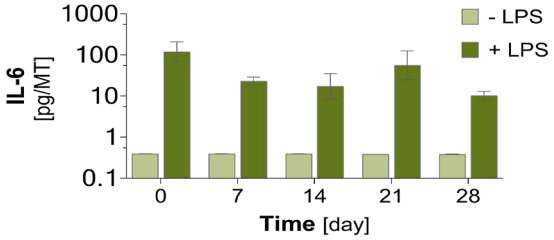


Exemplary data showing dose-dependent lactate release induced by phenformin, a drug that inhibits mitochondrial oxidative phosphorylation *in vivo*, after 7-day treatment in human liver microtissues.

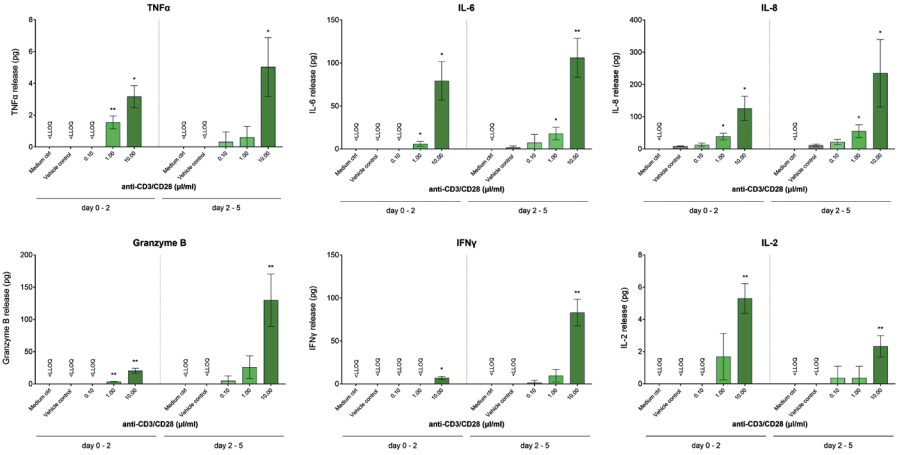
***3D InSight™ Species-Specific Liver Microtissues (Cynomolgus Monkey, Beagle Dog, Sprague-Dawley Rat, C57BL/6 mouse)**

Cytokine Release: Luminex (standard 6-plex: IFNγ, IL2, IL6, IL8, Granzyme B, TNFα) or specific cytokine ELISA

Assess the inflammatory potential of a compound when it is applied to human liver microtissues in the presence of the innate immune system (Kupffer cells). Cytokine Release measurements are available for human liver microtissues. Multiplexing is possible with all other biochemical assays.



Exemplary data showing IL-6 release in human liver microtissues treated with LPS for 48h, in cultures maintained for up to 29 days. IL-6 is measured with ELISA kit. n.d. = not detected



Exemplary data showing dose-dependent cytokine release induced by anti-CD3/CD28, a T-cell activator, in human liver microtissues co-cultured with PBMCs in 5-day treatment.

All-Around - One liver model through the entire drug discovery and development process

Predictive Toxicology
DILI Hazard Identification & Screens

Investigative Toxicology
DILI Mode of Action

Translation to man
Species-Specific DILI

OMICS

ANALYSE OMIC SIGNATURES TO UNRAVEL ADVERSE REACTIONS

- **TRANSCRIPTOMICS**
  - Low-input Bulk RNA-Seq
  - TempO-Seq RNA
- **PROTEOMICS***
- **BILE ACID PROFILING***
- **CYTOKINE PROFILING***
- **LIPID-MEDIATOR PROFILING***
- **LIPIDOMICS***

*proof of concept

MECHANISTIC STUDIES

TEST ADVERSE REACTIONS WITH SPECIALIZED EXPERIMENTAL SETUPS

REACTIVE METABOLITES

- **CYP450 CAUSALITY ASSAY**
  Co-treatment with ABT-1
  Readout: ATP, LDH
- **GLUTATHIONE CAUSALITY ASSAY**
  Co-treatment with BSO
  Readout: ATP, LDH

IMMUNOLOGICAL TOXICITY

- **INFLAMMATION CAUSALITY ASSAY**
  Co-treatment with LPS
  Readout: ATP, LDH
- **CYTOKINE PROFILING**
  Luminex (6-plex)

MITOCHONDRIAL FUNCTION ASSAY

- Readout: ATP, Lactate Release Assay

CHOLESTASIS

- **CANALICULAR SECRETION INHIBITION ASSAY**
  Measure of decreased signal of Cholyl-lysyl-fluorescein (CLF), a bile acid analogue
- **BILE ACID SECRETION ASSAY**
  Readouts: ATP, LDH, GCA/GCDCA quantification in supernatant using LC-MS

APOPTOSIS INDUCTION

- **CASPASE 3/7 ACTIVITY ASSAY**
- **CASPASE-CLEAVED KERATIN-18 ASSAY**

STEATOSIS INDUCTION ASSAY

- **TRIGLYCERIDE CONTENT ASSAY**

OXIDATIVE STRESS

- **ROS CAUSALITY ASSAY**
- **GSH OR GSH/GSSG ASSAY 18 ASSAY**

3D InSight™ | 3D *In Vitro* Liver Toxicology

Predictive Toxicology Services*

DILI Flag Assay - Human, 7 Days, 384-well Plate
DILI Flag Plus Assay - Human, 7 Days, 384-well Plate
DILI Assay - Human, 7 Days, 96/384-well Plate
DILI Assay - Human, 14 Days, 96/384-well Plate
DILI Assay - Human, 21 Days, 96/384-well Plate
DILI Assay - Human, 28 Days, 96/384-well Plate
DILI Assay - C57BL/6 Mouse, 7 Days, 96-well Plate
DILI Assay - C57BL/6 Mouse, 14 Days, 96-well Plate
DILI Assay - C57BL/6 Mouse, 21 Days, 96-well Plate
DILI Assay - C57BL/6 Mouse, 28 Days, 96-well Plate
DILI Assay - Beagle Dog, 7 Days, 96-well Plate
DILI Assay - Beagle Dog, 14 Days, 96-well Plate
DILI Assay - Beagle Dog, 21 Days, 96-well Plate
DILI Assay - Beagle Dog, 28 Days, 96-well Plate
DILI Assay - Cynomolgus Monkey, 7 Days, 96-well Plate
DILI Assay - Cynomolgus Monkey, 14 Days, 96-well Plate
DILI Assay - Cynomolgus Monkey, 21 Days, 96-well Plate
DILI Assay - Cynomolgus Monkey, 28 Days, 96-well Plate
DILI Assay - Sprague-Dawley Rat, 7 Days, 96-well Plate
DILIplus Assay - Human, 7 Days, 96-well Plate
DILIplus Assay - Human, 14 Days, 96/384-well Plate
DILIplus Assay - Human, 21 Days, 96/384-well Plate
DILIplus Assay - Human, 28 Days, 96/384-well Plate
DILIplus Assay - C57BL/6 Mouse, 7 Days, 96-well Plate
DILIplus Assay - C57BL/6 Mouse, 14 Days, 96-well Plate
DILIplus Assay - C57BL/6 Mouse, 21 Days, 96-well Plate
DILIplus Assay - C57BL/6 Mouse, 28 Days, 96-well Plate
DILIplus Assay - Dog, 7 Days, 96-well Plate
DILIplus Assay - Beagle Dog, 14 Days, 96-well Plate
DILIplus Assay - Beagle Dog, 21 Days, 96-well Plate
DILIplus Assay - Beagle Dog, 28 Days, 96-well Plate
DILIplus Assay - Monkey, 7 Days, 96-well Plates
DILIplus Assay - Cynomolgus Monkey, 14 Days, 96-well Plate
DILIplus Assay - Cynomolgus Monkey, 21 Days, 96-well Plate
DILIplus Assay - Cynomolgus Monkey, 28 Days, 96-well Plate
DILIplus Assay - Sprague-Dawley, 7 Days, 96-well Plate

Add On Services

- Solubility Assay
- Supernatant Collection and Storage (for three months)
- LDH Assay from collected and stored Supernatant (timepoints according to dosing)
- ALT Measurements from collected and stored Supernatant (timepoints according to dosing)
- Albumin ELISA from collected and stored Supernatant (timepoints according to dosing)
- Lactate Assay from collected and stored Supernatant (timepoints according to dosing)
- Caspase 3/7 activity assay from stored Supernatant (timepoints according to dosing)
- ccK18 from collected and stored Supernatant (timepoints according to dosing)
- Cytokine Release (Luminex or ELISA) from collected and stored Supernatant (timepoints according to dosing)
- Basic Written Report (including one post-study consultation with scientist)
- Regulatory-Friendly Report (including post-assay, 10 work hours of a scientist)
- Cmax Assessment Report
- Data Mining Package
- Fast Track Service

*These services can be performed on different modalities, including small molecules, oligonucleotides, siRNAs, ASOs,antibodies, ADCs:. Species-Specific Studies can be performed beyond 7-days for certain species. Ask our scientists or BD formore information. Additional Add-on Services available. Please inquire for details: **customerservice@insphero.com**

Sneak peek into our Investigative Toxicology Offerings

Ask for the corresponding brochure for more information

Investigative Toxicology - Transcriptomic Studies*

Basic Transcriptomic Analysis
Extended Transcriptomic Analysis
Low-Input Bulk RNA Transcriptomics - Human, 2 timepoints, 2 Test Concentrations, 96/384-well Plate
Low-Input Bulk RNA Transcriptomics - C57BL/6 Mouse, 2 timepoints, 2 Test Concentrations, 96-well Plate
Low-Input Bulk RNA Transcriptomics - Dog, 2 timepoints, 2 Test Concentrations, 96-well Plate
Low-Input Bulk RNA Transcriptomics - Monkey, 2 timepoints, 2 Test Concentrations, 96-well Plate
Low-Input Bulk RNA Transcriptomics - Sprague-Dawley, 2 timepoints, 2 Test Concentrations, 96-well Plate
TempO-Seq RNA Transcriptomics - Human, 2 timepoints, 2 Test Concentrations, 96/384-well Plate
TempO-Seq RNA Transcriptomics - C57BL/6 Mouse, 2 timepoints, 2 Test Concentrations, 96-well Plate

Investigative Toxicology - Mechanistic Studies*

CYP450 Causality Assay - Human and Species-Specific, 7 days, 96/384-well Plate
Glutathione Causality Assay - Human, 7 days, 96/384-well Plate
ROS Causality Assay - Human, Monkey, Dog, 7 days, 96/384-well Plate
Inflammation Causality Assay - Human, 7 days, 96/384-well Plate
Mitochondrial Function Assay - Human and Species-Specific, 7 Days, 96/384-well Plate
Canalicular Secretion Inhibition Assay - Human, 7 days, 384-well Plate
Bile Acid Secretion Assay - Human, 7 days, 96/384-well Plate
Caspase 3/7 Activity Assay - Human and Species-Specific, 7 days, 96/384-well Plate
Caspase-Cleaved Keratin-18 Activity Assay - Human, 7 days, 96-well Plate
Steatosis Induction Assay - Human, Rat and Dog, 7 Days, 96/384-well Plate
GSH/GSSG Assay - Human , 7 days, 384-well Plate

*These services can be performed on different modalities, including small molecules, oligonucleotides, siRNAs, ASOs, antibodies, or ADCs. Species-specific studies can be performed beyond 7-days for certain species. Ask our scientists or BD for more information. Additional Add-on Services available. Please inquire for details:
customerservice@insphero.com

Publications

Drug-Induced Liver Injury (DILI) Prediction

Screen and assess hazard and risk for hepatotoxicity and get human-relevant insights.

- Fäs et al., Toxicol Sci. 2025. PMID: 39397666
- Proctor et al., Arch Toxicol. 2017. PMID: 28612260
- Albrecht et al., Arch Toxicol. 2019. PMID: 31250071
- Shrimali et al., Drug Disc. 2025. PMID: 40803573

Environmental Toxicology

Evaluate MOAs, potency ranking, and hepatotoxic effects of environmental contaminants such as PFAS.

- Rowan-Carroll et al., Toxicol Sci. 2025. PMID: 40037795
- Rowan-Carroll et al., Toxicol Sci. 2021. PMID 33772556
- Kermanizadeh et al., Part Fibre Toxicol. 2019. PMID: 31739797
- Baumert et al., Nature. 2025. PMID: 39006440
- Yeisley et al., Biochem. & Biophys. Reports. 2025. PMID: 40832469
- Kermanizadeh et al., Part Fibre Toxicol. 2014. PMID: 25326698

Antiviral Drug Efficacy Testing

Model viral replication and assess cytopathology and drug efficacy against hepatotropic and systemic viruses.

- Chaput et al., Antiviral Res. 2025. PMID: 40360123

Model Description and Characterization

Investigate compound mechanisms, rank biological activity, and profile functional responses in physiologically relevant liver models.

- Messner et al., App. In Vitro Toxicol. 2018. PMID: 32953943
- Fruhwürth et al., Histochem Cell Biol. 2014. PMID: 25059650
- Alepee et al., ALTEX. 2014. PMID: 25027500
- Messner et al., Arch Toxicol. 2013. PMID: 23143619

Regenerative Medicine & Cell Therapy

Evaluate engraftment and therapeutic potential of hepatic progenitor cells, supporting the development of cell-based therapies and liver regeneration strategies.

- Alipio et al., Commun Biol. 2025. PMID: 40640572

Species-Specific Translational Research

Compare human, rat, and dog liver microtissues to study species-specific toxicity mechanisms and support translational risk assessments for better outcomes.

- Bajaj et al., Toxicol Sci. 2025. PMID: 39886943
- Plummer et al., Toxicol. Rep. 2019. PMID: 31673501
- Odermatt et al., Tissue Sci Eng. 2013. DOI: 10.4172/2157-7552
- Elcombe et al., Toxicol. Rep. 2025. PMID: 41050184

Mechanistic Toxicology & Omics

Uncover the underlying pathway-level insights into compound-induced hepatotoxicity using transcriptomic, proteomic, and imaging technologies.

- Bajaj et al., Toxicol Sci. 2025. PMID: 39886943
- Shinozawa et al., Toxicol Sci. 2025. PMID: 39786842
- Bruderer et al., Mol Cell Proteomics 2015. PMID: 25724911
- Hendriks et al., Sci Rep. 2016. PMID: 27759057
- Borgström et al., App. In Vitro Toxicol. 2023. DOI: 10.1093/avit.2023.0004
- Subbiah et al., Clin Cancer Res. 2023. PMID: 37265425
- Kermanizadeh et al., Sci Rep. 2019. PMID: 31086251
- Paech et al., Arch Toxicol. 2017. PMID: 28536862

AI-based Prediction

Apply 3D liver models to generate a high-quality, large multi-modal dataset to train neural networks for developing AI-based prediction models.

- Dubinsky et al., Front. Bioeng. Biotechnol. 2025. DOI: 10.1101/2025.02.09.642246

Multi-organ translational studies

Integrate liver microtissues in an organ-on-a-chip system and simulate multi-organ studies, ideal for drug-drug interactions & translational studies.

- Wu et al., Biomed Pharmacother. 2025. PMID: 40179733
- Lohasz et al., Adv Biosyst. 2020. PMID: 33073544

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