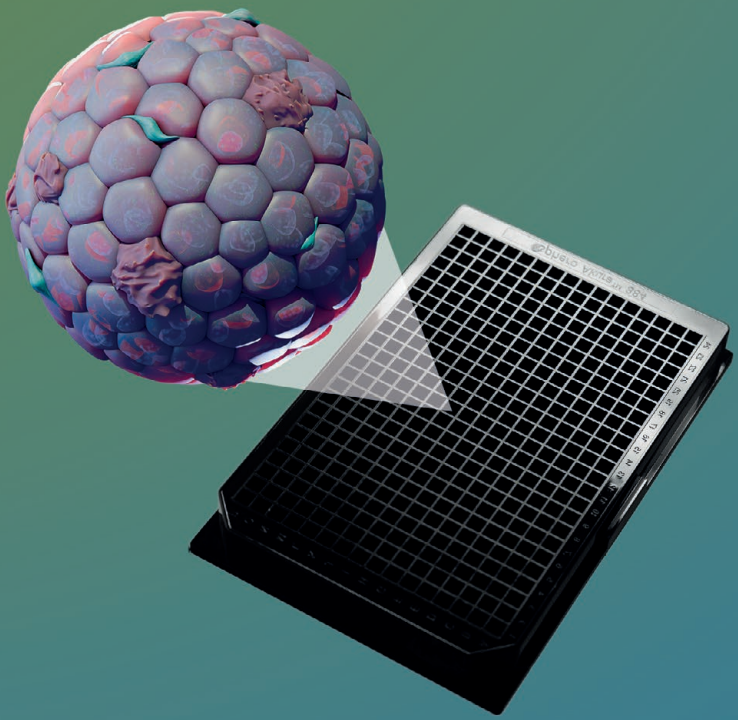
insphero

The Model of Excellence™

3D InSight™ *in vitro* Liver Toxicology

Predictive Toxicology Services



Confidently predict DILI with 3D InSight™ Liver Microtissues:
delivering early, reliable data to de-risk your pipeline

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

Confidently predict and identify potential hepatotoxic liabilities early with 3D InSight™Liver Microtissues, delivering reliable DILI data to de-risk your pipeline.

Standardized testing that delivers high-quality, reproducible, and actionable data, benchmarkable against a large dataset of FDA-approved drugs (Fäs et al., Toxicol. Sci., 2025), enabling you to make confident go/no-go decisions, de-risk your development programs, and faster bring safer drugs to market.

Reliable DILI prediction with physiological 3D liver models

Achieve accurate DILI predictions with 3D InSight™ Liver Microtissues, physiologically relevant coculture liver models composed of primary hepatocytes and non-parenchymal liver cells from human or preclinical species.

With four weeks of sustained viability and functionality, these models closely mimic *in vivo* liver physiology, enabling early and thorough evaluation of your drug candidates' toxicity.

Faster go/no-go decisions with standardized cytotoxicity DILI testing

Accelerate your drug discovery with our standardized and high-throughput cytotoxicity DILI assay. We offer weekly human and monthly animal DILI testing, evaluating 7 concentrations of your compound in 4 biological replicates with up to 28 days of treatment.

You'll receive a comprehensive analysis report within 10 working days after completion of experimental work, streamlining fast decision-making and keeping your projects moving forward.

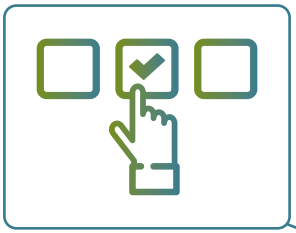
Benchmark drug candidates against published peer-reviewed DILI reference database

Make confident go/no-go decisions with reference data from hundreds of FDA-approved drugs. We continuously screen compounds using our standardized DILI assays and publish the results in leading scientific journals, building a comprehensive database to benchmark your findings.

- 7-day DILI assessment in Akura™ 384 Spheroid Microplate (Fäs et al., Toxicol .Sci., 2025)
- 14-days DILI assessment in Akura™ 96 Spheroid Microplate (Proctor et al., Arch. Toxicol., 2017)

How to get started with our DILI Testing service

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

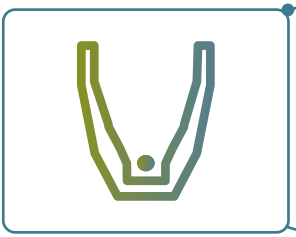


Select the right assay for your program

From early-stage DILI hazard identification to advanced multi-parametric readouts, choose a solution aligned with your discovery or preclinical strategy.

Simplified compound logistics

Ship your drug candidates with ease. We coordinate logistics and prepare 3D liver microtissues in parallel to keep timelines on track.



Expert-driven *in vitro* DILI testing

Our scientific team executes your study with optimized dosing strategies, validated workflows, and robust readouts for high-confidence results.

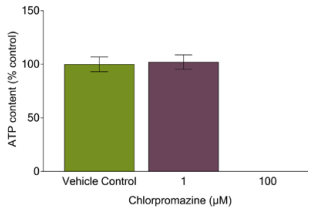
Comprehensive, decision-ready reporting

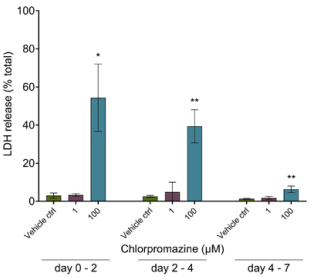
Receive a detailed, publication-grade report with actionable insights designed to support internal reviews, regulatory submissions, and portfolio decisions within 10 business days after completion of experimental work.

DILI Flag / DILI Flag Plus

Accelerate Hit-to-Lead phase with our high-throughput early screening assay for rapid detection and flagging of highly cytotoxic compounds

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) DILI Flag Plus: ATP (endpoint) and LDH activity leakage: 3 timepoints (7-day: day 2, 5 & 7; 14-day: day 5, 9, & 14)
Quality Control Criteria	100 uM Chlorpromazine: ▪ Complete loss of cellular ATP; ▪ DILI Flag Plus: Statistically significant ($p<0.05$) LDH activity leakage 1 uM Chlorpromazine: no statistically significant cellular ATP reduction or LDH leackage
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
Note	Minimal number of compounds per submission: 8



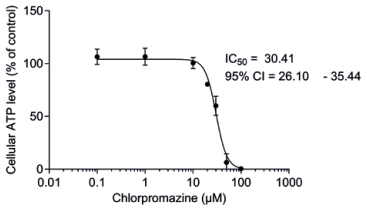


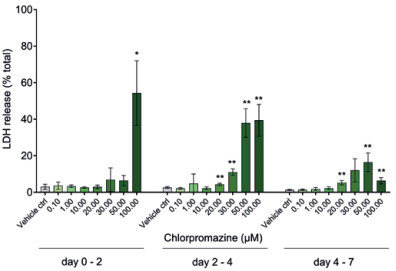
Exemplary data showing cytotoxicity induced by chlorpromazine in human liver microtissues in 7-day DILI Flag Plus assay (left: ATP; right LDH leakage).

DILI / DILI Plus

Accelerate lead optimization phases by rank ordering the cytotoxicity of your lead compounds and benchmark drug candidates against published peer-reviewed DILI reference database

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-day
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 DILI Assay Data ($IC50_{ATP}$) into Actionable Insight

Human DILI Assay

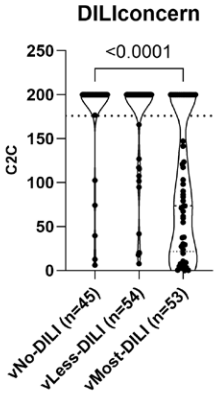
ALL MODALITIES

Safety ranking of Lead Optimization selection

SMALL MOLECULES

Evaluate safe exposure for orally administered small molecules

- **7-day DILI assay in 384-well plate**
  C2C ratio ($IC50_{ATP}$/ Cmax) < 176 distinguishes hepatotoxic from safe 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

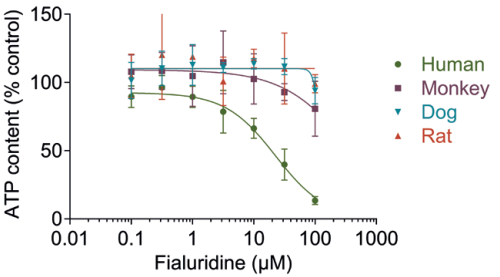


Adapted from Fäs et al., Toxicol. Sci., 2025. IC50ATP/ Cmax of 152 FDA-approved orally administered orally admisistered drugs is significantly different between no-DILI and Most-DILI concern drugs

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

DILI and DILI Plus Services can be multiplexed with several biomarkers measured in the collected supernatant, providing a kinetic overview of the investigated mechanism

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.

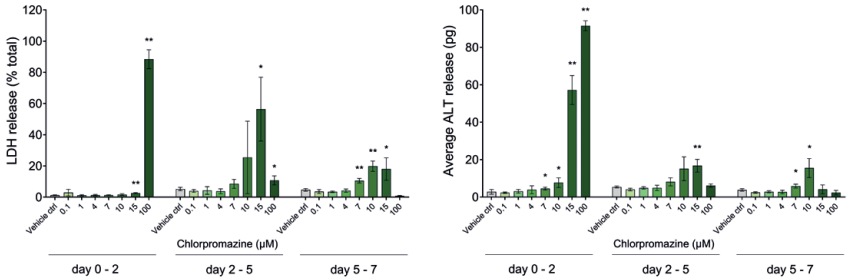
	Assay Type				
Event/ Function	**Biomarker**	**Species**	**Clinical Marker**	**Species**	**Volume Required**
Loss of membrane integrity (necrosis)	**LDH assay**	Human, Species-Speciefic Models*	**ALT ELISA**	Human, Rat	LDH: 5ul, ALT 96: 25ul, ALT 384: 12.5ul
Apoptosis	**Caspase 3/7 Activity Assay**	Human, Species-Speciefic Models*	**ccK18 ELISA**	Human	Caspase 3/7: 20 µl Human ccK18: 20µl
Hepatocyte functionality	**Albumin Human: 5ul: Rat: 10ul; Dog 5 ul; Monkey 5 ul**	Human, Species-Speciefic Models*	**n.a.**		Human: 5ul, Rat: 10ul, Dog 5 ul, Monkey 5 ul
Mitochondrial dysfunction	**Lactate Detection Assay**	Human, Species-Speciefic Models*	**n.a.**		Lactate Assay: 2.5 µl
Inflammation	**Customizable cytokine panel (Luminex) or specific cytokine ELISA**	Human	**n.a.**		Luminex 96: 25 ul; 384: 12.5 ul

***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

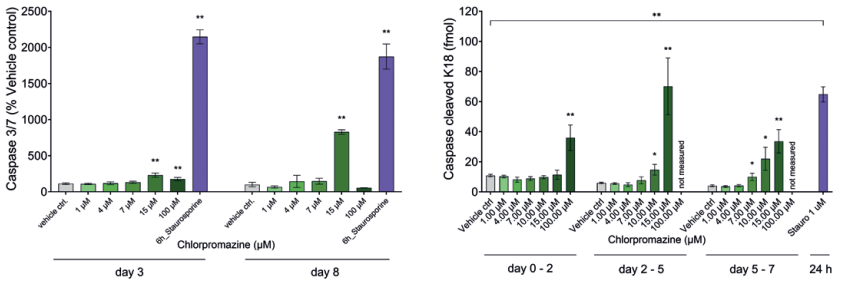
Measurement of leakage markers provides a kinetic overview of cytotoxicity and necrosis induced by the test item. The LDH Assay is available in human and species-specific microtissues*. ALT ELISA is available in Human and Sprague Dawley Rat Liver Microtissues Multiplexing is possible with all other biochemical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



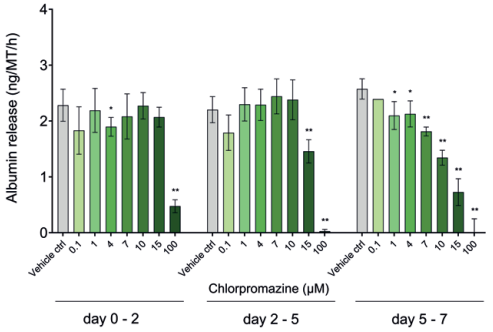
Exemplary data showing onset of apoptosis induced by Chlorpromazine in human liver microtissues in 7-day treatment (left: Caspase 3/7 activity; right: ccK18 ELISA)

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

Other Liver Functionality Markers

Hepatocytes Functionality: Albumin ELISA

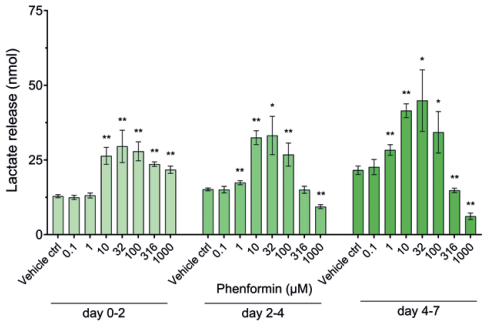
Hepatocytes in liver microtissues maintain stable albumin synthesis for up to 28 days. A decrease in albumin production after treatment indicates potential hepatotoxicity. Albumin ELISA is available in human and species-specific microtissues.



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

Mitochondrial Functionality Assay: Lactate Release Assay

Lactate Release Assay evaluate 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.



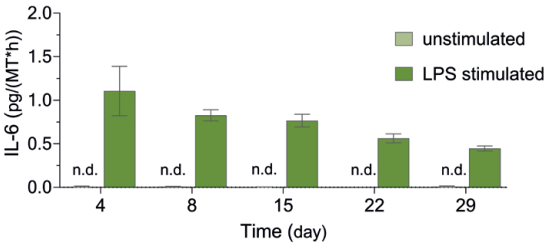
Exemplary data showing dose-dependent lactate release induced by phenformin, a drug that inhibits mitochondrial oxidative phosphorylation *in vivo*, after 7-day treatment in human liver microtissues.

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

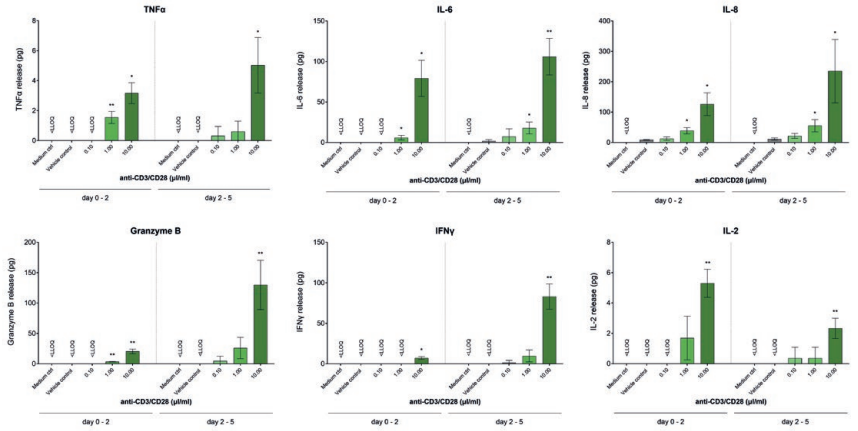
Inflammation Induction

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 is applied to Human Liver Microtissues in 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, T-cells activator, in human liver microtissues co-cultured with PBMCs in 5-day treatment.

Additional 3D InSight™ Liver Toxicology Services

All-Around - One liver model for all your liver safety assessment

Predictive Toxicology
DILI Hazard Identification

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
  (optional: GSH or GSH/GSSG assay)
- **ROS CAUSALITY ASSAY**
  Co-treatment with NAC
  Readout: ATP, LDH
  (optional: GSH or GSH/GSSG assay)

IMMUNOLOGICAL TOXICITY

- **INFLAMMATION CAUSALITY ASSAY**
  Co-treatment with LPS
  Readout: ATP, LDH
  (optional: IL-6 ELISA)
- **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
- **BILE ACID CAUSALITY ASSAY**
  Co-treatment with physiological mix of bile acids. Readout: ATP, LDH

APOPTOSIS INDUCTION

- **CASPASE 3/7 ACTIVITY ASSAY**
- **CCK18 ASSAY**

STEATOSIS INDUCTION ASSAY

- **TRIGLYCERIDES CONTENT 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 - Beagle Dog, 7 Days, 96-well Plate
DILI Assay - Cynomolgus Monkey, 7 Days, 96-well Plate
DILI Assay - Sprague-Dawley Rat, 7 Days, 96-well Plate
DILIplus Assay - Human, 7 Days, 96/384-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 - Dog, 7 Days, 96-well Plate
DILIplus Assay - Monkey, 7 Days, 96-well Plates
DILIplus Assay - Sprague-Dawley Rat, 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 storedSupernatant (timepoints according to dosing)
Albumin ELISA from Ccollected 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 Assay 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 workhours 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 for more information. Additional Add-on Services available. Please inquire for details: **customerservice@insphero.com**

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

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 Rat, 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 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

Bile Acid Causality Assay - Human, 7 days, 96/384-well Plate

Caspase 3/7 Activity Assay - Human and Species-Specific, 7 days, 96/384-well Plate

ccK18 Assay-18 Activity Assay - Human, 7 days, 96-well Plate

Steatosis Induction Assay - Human, Rat and Dog, 7 Days, 96/384-well Plate

*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 for more information. Additional Add-on Services available. Please inquire for details: **customerservice@insphero.com**

NOTES

inSphero

The Model of Excellence™

InSphero is the only 3D in vitro model company with the platform and expertise to modernize drug discovery and drug safety testing in a way that empowers researchers to reach their full potential. Our innovative spheroid-based models allow for more effective, safe therapies to reach the clinic through truly superior robustness, reliability, reproducibility, and scalability. InSphero's goal is to inspire the next generation of breakthrough therapies through customer devotion, commitment to innovation, and the model of excellence.

LEARN HOW INSPHERO IS MODERNIZING DRUG DISCOVERY HELPING TO FUEL A NEW ERA OF BREAKTHROUGH MEDICINES.

Don't wait, let's talk!

For questions or orders please scan the QR code or

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