insphero
Biomimetic Microtissue Technology

Agilent Technologies

3D InSight™ Service
Mitochondrial Toxicity Testing

Leverage advanced, organotypic 3D liver microtissues to assess mitochondrial liabilities.

- **Perform label-free assessment of oxygen consumption rate (OCR)** by combining 3D InSight™ Human Liver Microtissues with the Agilent XF^{e}96 Analyzer
- **Identify mitochondrial liabilities with high sensitivity and specificity** in a primary 3D liver model with 6X higher spare respiratory capacity (SRC) than 2D monolayer hepatocyte cultures
- **Study long-term drug exposure effects** with repeat dosing in a highly predictive two-step assay

3D InSight™ Mitochondrial Toxicity Testing uses a robust and highly predictive two-step assay to combine organotypic 3D liver microtissues with state of the art label-free mitochondrial function analysis on the Agilent XF^{e}96 Analyzer. The longer *in vitro* lifespan of 3D InSight™ Human Liver Microtissues enables extended culture and flexible drug exposure times (2 to 14 days) with repeat dosing. The assay decouples drug treatment and assay phases with a discrete transfer of spheroids from GravityTRAP™ culture plates to the Agilent XF^{e}96 platform for OCR analysis (Figure 1), and parallel testing of replicates for cell viability (microtissue ATP content, Promega CellTiter-Glo®).

The assay capitalizes on the 6X greater spare respiratory capacity (SRC) of primary human hepatocytes cultured as 3D microtissues as compared to when grown in 2D monolayer culture (Figure 2). A drop in SRC, defined as the difference between maximum and baseline oxygen consumption rate (OCR), is one of the first direct consequences of mitochondrial impairment.

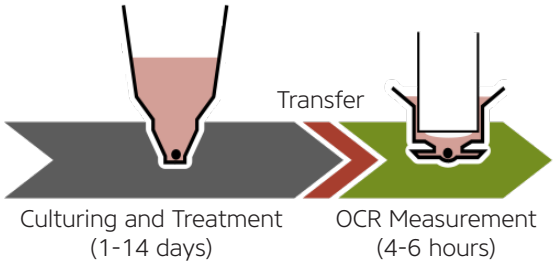

Figure 1: *Two-step workflow for 3D InSight™ Mitochondrial Toxicity Testing includes the initial drug exposure phase followed by independent OCR assessment on the Agilent XF^{e}96 Analyzer.*

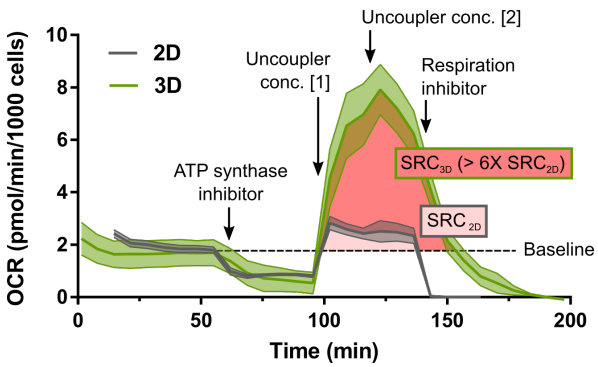

Figure 2: *SRC assessment in primary human hepatocytes grown in 3D (green) vs. 2D (gray). Curves represent means and SD. The 6X greater SRC of 3D microtissues (dark pink) more closely reflects that of* in vivo *liver.*

Compound classification with mitochondrial toxicity testing data

Dose-dependent changes in cell viability (ATP) and SRC are used in combination to determine if mitochondrial impairment is the primary toxicological mechanism or a secondary effect. The ratio of IC_{50}SRC to IC_{50}ATP classifies compounds based on their potential hepatotoxicity and mitochondrial liability (Figure 3, Table 1).

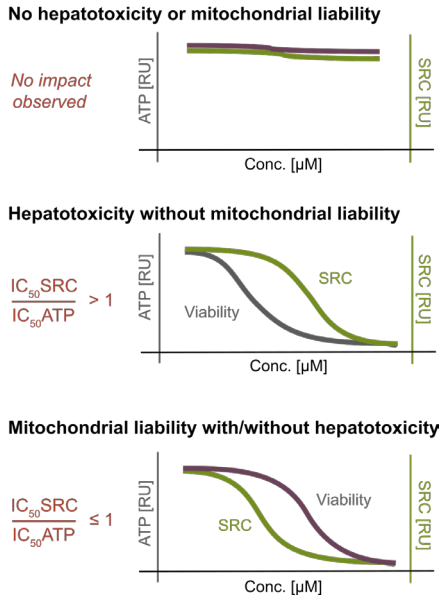

Figure 3: *Compound classification scheme.*

Table 1: *3D InSight™ Mitochondrial Toxicity Testing Service classification results*

Compound Class	Compound Name	Known Association with Mitotoxicity	Results		Classification	
			IC_{50}ATP [µM]	IC_{50}SRC [µM]	DILI	Mitochondrial Liability
Cardiovascular Drugs	Amiodarone	Y	> 100	~ 24.9	+	+
	Bosentan	N	> 250	257.1	-	-
	Perhexiline Maleate	Y	10.07	~ 7.6	+	+
	Ximelagatran	N	> 250	> 250	-	-
Anti-diabetic Drugs	Metformin HCl	N	> 1000	774.2	-	-
	Troglitazone	Y	41.49	19.7	+	+
CNS Drugs	Buspirone	N	> 500	> 500	-	-
	Entacapone	N	249.7	334.0	-	-
	Nefazodone	Y	~ 31.8	18.6	+	+
	Tolcapone	Y	57.01	45.3	+	+
Analgesic Drugs	Acetaminophen	Y	6948	3801	+	+
Anti-inflammatory Drugs	Diclofenac	Y	> 250	177.0	+	+
Antiviral Drugs	Fialuridine	Y	> 100	> 100	-	-

Notes: *Compound classification for this test set achieved:* ***88% Sensitivity*** *(7 of 8 correctly predicted);* ***100% Specificity*** *(5 of 5 correctly predicted).*

3D InSight™ Mitochondrial Toxicity Testing

Catalog number
SP-02-210-01

Model system
3D InSight™ Human Liver Microtissues (MT-02-302-11), qualified for OCR analysis

Standard experimental set-up
- ***Phase I.*** Dose range-finding for cell viability: 2 days
- ***Phase II.*** OCR analysis on Agilent XF^{e}96 and cell viability: 2 days

Number of dosings
2 (Days 0 and 1)

Tested compound concentration
- ***Phase I.*** 7-point dose-response curve
- ***Phase II.*** 7-point dose-response curve

Positive control compound
Amiodarone (complex I + II inhibitor)

Endpoints
- ***Phase I.*** Dose-dependent intra-tissue ATP content (CellTiter-Glo®, Promega Corp.)
- ***Phase II.*** Dose-dependent spare respiratory capacity (Agilent XF^{e}96) and intra-tissue ATP content (CellTiter-Glo®)

Data analysis
- Dose-response for cell viability (IC_{50}ATP) for Phase I and II
- Dose response for spare respiratory capacity (IC_{50}SRC)
- Analysis of compounds regarding mitochondrial liability based on viability and SRC dose response curves
- Raw data on request
- Written report including material and methods, compound information, graphs, and results summary

Customization
- Drug exposure times for Phase II can be customized (up to 14 days). The number and timing of repeat dosings will vary based on the length of drug exposure

Request our white paper to learn more.

Visit www.insphero.com/services for more details about this and other 3D InSight™ Services, or contact our local branch offices and authorized distributors.

IS-500-0022-01-B

InSphero AG
Schlieren, Switzerland
✆ +41 44 515 04 90

InSphero Inc.
Brunswick, ME, USA
✆ +1 800-779-7558

InSphero Europe GmbH
Waldshut, Germany
✆ +49 7751 30496650

info@insphero.com
www.insphero.com

Authorized Distributors
www.insphero.com/distributors

InSphero is ISO 9001:2008 certified

All rights reserved, © 2017 InSphero AG. 3D InSight is a trademarks of InSphero AG. CellTiter-Glo is a registered trademark of Promega Corp. For life science research only. Not for use in diagnostic procedures.