Akura™ 3D SureKit

Product Manual

PM006, July 2023

Contents

Introduction

The Akura™ 3D SureKit provides an all-in-one solution to generate and culture organotypic 3D spheroids from primary human hepatocytes.

It offers a variety of unique characteristics to make your 3D work faster, more flexible, cost efficient and more convenient:

- Ease of use and high reproducibility by using pre-qualified cells and thoroughly tested protocols
- Flexibility to perform your assays at your convenience, you can store the kit until just before performing your assays.
- Options to modify hepatocytes before or during the aggregation process (e.g. by viral transduction methods or gene-editing systems)
- Possibility to tailor spheroid size by varying seeding cell numbers
- Option to customize spheroid composition by introducing additional cell types
- Advantage to benchmark generated liver spheroids to characterization data (morphology, cell health, functionality and sensitivity towards a panel of standard DILI compounds)

The production of human liver spheroids using the Akura™ 3D SureKit is truly straightforward. The kit is designed to make 3D research with primary hepatocytes easily accessible to those interested in working with an advanced *in vitro* model, while keeping entry costs for equipment needed and time invested minimal.

The Akura™ 3D SureKit contains:

- Primary human Hepatocytes[1] thoroughly tested to form 3D spheroids [2]
- The best-in-class 3D cell culture compatible Akura™ Plates, which guarantee homogenous aggregation of cells, minimal tissue loss during media exchanges[3] and an optimized geometry designed to minimize media evaporation
- InSphero proprietary aggregation and culture media which are essential for generating and maintaining the spheroids
- Access to QC data and protocols[4]

[1] *The Akura™ 3D SureKit contains validated hepatocytes from single donors. If you would like to work with more complex models or microtissues from pooled donors, please check our 3D InSight™ products or talk to our customer service representatives.*

[2] *Hepatocyte spheroids can be consistently produced if provided protocol is exactly followed*

[3] *Up to 30% of spheroids are lost with each media exchange when using other commercially available plates, leading to irreproducible results and increased research costs*

[4] *Characterization data consists of tissue morphologies, viabilities, organotypic functionality and compound sensitivity data.*

Figure 1: Akura™ 3D SureKit Components

Background

The architecture and arrangement of hepatocytes *in vivo* is sophisticated and complex. *In vivo*, hepatocytes form tight cell-cell connections among each other which are critical for proper function and polarity (Schulze et al., 2019). Each hepatocyte has an apical domain as well as two basolateral domains, where there is crucial alignment of uptake and efflux transporters responsible for transport of proteins and bile acids, as well as drug transport. When plated on collagen-coated plates, it is extremely difficult for primary hepatocytes to achieve the high confluency needed to recapitulate this *in vivo* architecture. Decreased cell-cell connections in plated hepatocytes are accompanied by less transporter expression. Other major hepatocyte functions are compromised as well, including significant downregulation of metabolic enzymes and albumin secretion (Bell et al., 2018). Therefore, it has become widely accepted in liver research that 3D models are superior to 2D models. Allowing liver cells to self-aggregate in a scaffold-free environment results in stronger cell-cell connectivity, realignment of crucial transporters, and retention of metabolic enzymes (Messner et al., 2013). All of these critical hepatocyte functions can affect the potential toxicity of a therapeutic, so it is critical that *in vitro* models reflect *in vivo* liver for toxicity research. Akura™ Assay Ready plates of primary hepatocytes (cocultured with nonparenchymal cells) have been proven to predict double the number of true DILI compound toxicity compared to the same hepatocyte lot plated in 2D (Proctor et al., 2017). This model is extremely beneficial for DILI

screening with simple biochemical endpoints such as ATP and LDH, as well as deep mechanistic studies into different types of toxicity (fibrosis, necrosis, apoptosis, steatosis, hepatitis, etc.).

While there is significant convenience in receiving highly QC'd liver spheroids from InSphero, there are some research applications where it can be advantageous to produce hepatic spheroids in your own lab. For example, gene editing through siRNA, AAV, CRISPR, etc., *prior* to aggregation will likely result in greater transfection/transduction efficiency than post aggregation. Furthermore, a kit with the necessary plates and hepatocytes will allow manipulation of different *quantities* of hepatocytes, as well as the possibilities to add in *additional* cell types of interest for specific research questions. Thus, the Akura™ 3D SureKit has been created for customer convenience, with the same quality expected from InSphero products. Potential applications include hepatocyte biology research, genetic editing, hepatotoxicity, steatosis research, etc. This kit includes everything necessary for creating quality hepatocyte spheroids in your own lab, in a cost-effective manner.

Components

Materials included

1. 3 x Akura™ 96 Spheroid Microplates
2. 1 x Akura™ Tilting Stand
3. Primary human hepatocytes, cryopreserved (> 1 million per vial)
4. 40 ml Aggregation base medium stored at 4 °C
5. 12.5 ml Aggregation supplement stored at -20 °C
6. 200 ml Maintenance base medium stored at 4 °C
7. 10 ml Maintenance supplement stored at -20 °C
8. Quick start guide

Additional materials required

1. Serological pipettes (preferentially wide-bore)
2. 15 ml Falcon tubes
3. 8- or 12-channel pipette (e.g. Viaflo 10-300 µl, Integra Biosciences)
4. Medium reservoir for multichannel pipettes
5. Microplate centrifuge
6. Humidified CO_2 incubator 37 °C
7. Water bath
8. Inverted microscope with 5x/10x objective

Protocol for generating 3D primary human hepatocyte spheroids

Upon Arrival

IMPORTANT:

Open pouch and transfer cryo vial with primary hepatocytes to liquid nitrogen immediately upon arrival.

Preparation

1. Prior to seeding, pre-warm the cell aggregation base medium.
2. Thaw aggregation medium supplement in a water bath set to 37 °C.
3. Wipe pre-warmed aggregation medium bottle and aggregation supplement tube with 70% EtOH and transfer to sterile working area.
4. Pipet contents of aggregation medium supplement to the aggregation base medium and mix.
5. Wipe the Akura™ Plate bag with 70% EtOH before opening.
6. Carefully open the bag under sterile working conditions and take out the Akura™ Plates.

Thawing

1. Remove vial with hepatocytes from cryo storage and thaw rapidly in a 37 °C waterbath until only a small ice chip remains in the center of the vial.
2. Immediately wipe the vial with 70% EtOH and transfer it to the sterile working area.
3. Aspirate 4 ml of complete aggregation medium in a 5 ml serological pipet (if available use a wide bore pipette).
4. Then slowly aspirate thawed cell suspension.
5. Dispense 1.5 ml of this 4.5 ml into a 15 ml Falcon tube.
6. Wash the cryo-vial by adding 1.5 ml from the suspension in the pipet to the vial. Re-aspirate the 1.5 ml into the pipet. Transfer 1.5 ml to the Falcon tube (point 6).
7. Repeat step 6 with the remaining volume in the pipette.
8. Add additional 8 ml of complete aggregation medium to the Falcon tube (point 5).
9. Gently tilt the Falcon tube containing the cell suspension 5 times.
10. Centrifuge Falcon tube at **80 RCF for 5 minutes.**

Hepatocyte seeding

1. Carefully remove **all** the supernatant.
2. Dispense 5 ml of aggregation medium to cells.
3. Resuspend pellet carefully by dislodging it with a 10 ml serological pipette (if available use a wide bore pipette) and very gently aspirate and dispense the suspension 2-3 times.
4. Depending on the desired spheroid size, prepare the desired volume of aggregation medium (M) in a 50ml Falcon tube (see Table 1).
5. transfer desired volume of cell suspension (S) to Falcon tube containing aggregation medium (Table 1).

Table 1 :

Seeding volumes

Spheroid size (diameter)	Cell number/MT	Volume cell suspension (S)	Volume aggregation medium (M)
~220 µm	750	1.3 ml	23.7 ml
~250 µm	1150	2.0 ml	23.0 ml
~300 µm	2500	4.3 ml	20.7 ml

6. Close Falcon tube and invert it gently invert it 2-3 times.
7. Transfer suspension to a reagent reservoir

IMPORTANT:

For uniformity of spheroids, it is essential to assure a homogeneous distribution of the hepatocytes by gently pipetting up and down the cell suspension in the reservoir prior to seeding into the Akura™ 96 Plate.

8. Using the multi-channel pipette, gently (~30 µl/sec) add 70 µl of the cell suspension per well to the Akura™ Plates by placing the pipette tips far into the wells (Figure 2). Between each plate, repeat the pipetting up and down step in the reservoir to mix the suspension.
9. Place the lids on the Akura™ Plates and spin in a microtiter-plate centrifuge for 60 seconds at 200 RCF.
10. Following centrifugation, place the plates on the supplied Akura™ Tilting Stand in a humidified CO_2 incubator at 37 °C for 4 days (see Figure 3 and Appendix D for technical details).

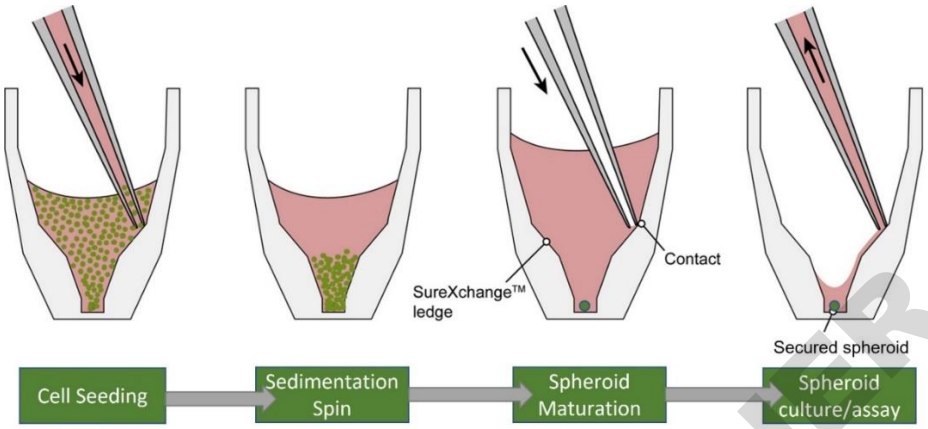


***Figure 2**: Spheroid formation in the Akura™ 96 Plate begins with initial seeding of hepatocyte cells in suspension, followed by a brief spin to sediment cells. Following spheroid maturation, the SureXchange™ ledge of the tapered well facilitates medium exchange and compound dosing without disturbing or losing the spheroid.*

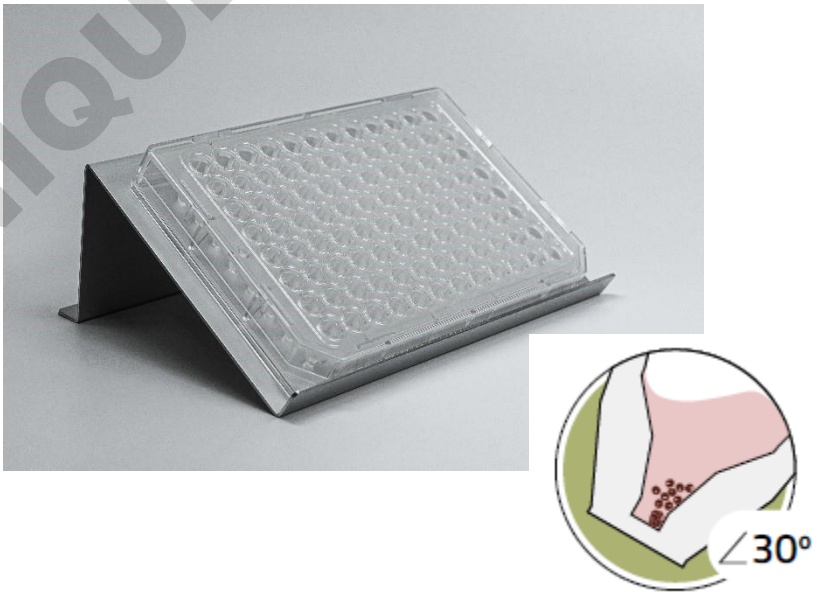


***Figure 3**. Akura™ Plate containing seeded cell suspension positioned on the tilting stand. The tilted positioning allows for a more homogenous spheroid aggregation. Up to three plates can be stacked on the tilting stand.*

Medium exchange

1. After 4 days, remove plates from incubator and inspect under microscope. Spheroidal aggregates with distinct morphology should have formed (Fig. 4).
2. Pre-warm maintenance base medium and thaw maintenance medium supplement in a water bath set to 37 °C.
3. Wipe pre-warmed maintenance medium bottle and maintenance supplement tube with 70% EtOH and transfer to sterile working area.
4. Pipet contents of maintenance medium supplement to the maintenance base medium and mix.
5. Replace the aggregation medium on the spheroids by maintenance medium. The special Akura™ 96 Plate design allows routine medium exchange for longer-term cultivation without the risk of spheroid loss. The SureXchange™ ledge at the inside wall of the well serves as an anchoring point for the pipette tip.
6. Place the pipette tip at the ledge of the well (Fig. 5, left).
7. Remove the medium at low pipetting speed (<30 µl/sec) by aspirating an excess of volume. A minimal volume of ~5-7 µl medium will remain in the well.
8. Add 70 µl of fresh medium by placing the pipette tip at the ledge (Fig. 5, right). Use a dispensing rate <50 µl/sec.

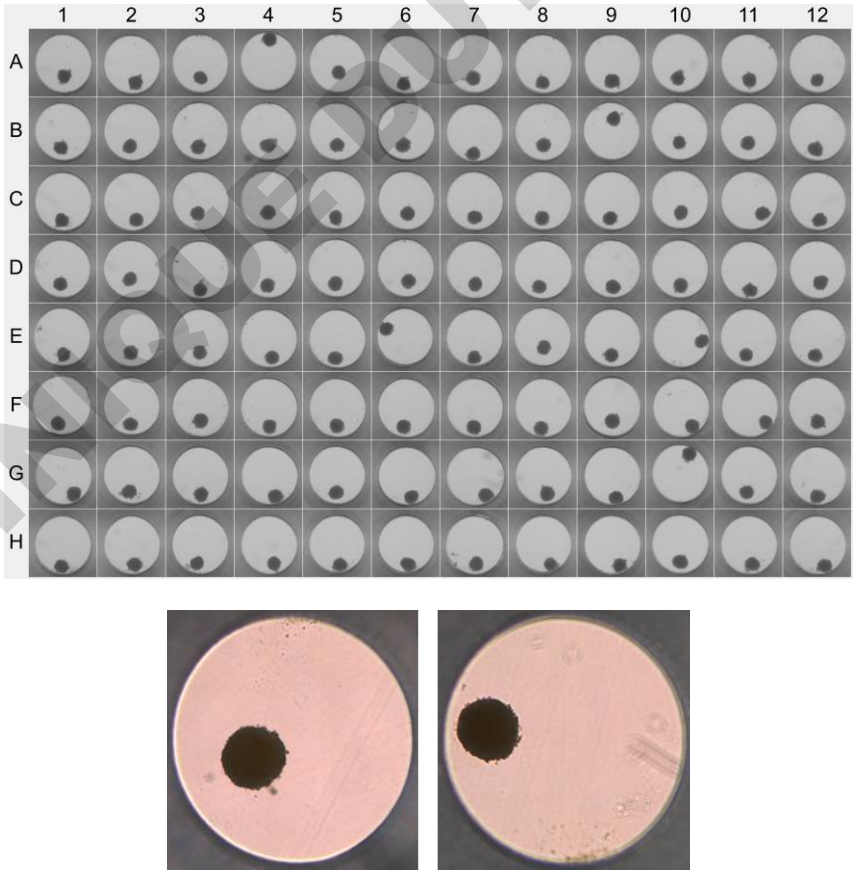


Figure 4: Plate-scan of aggregated liver spheroids (left). Phase contrast micrographs of two formed spheroids after aggregation (right)

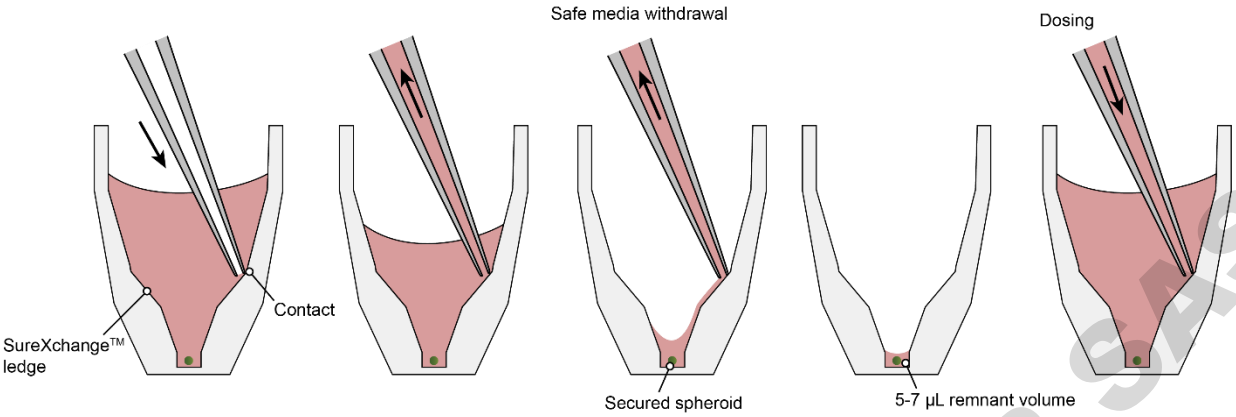


Figure 5. Medium exchange in the Akura™ Plate. Left: Medium removal with the pipette tip placed at the ledge of the well. Right: Medium addition.

IMPORTANT:

When using automated liquid handling devices, an off-center alignment of the vertical pipette tip will achieve the same affect. We recommend regular medium exchanges every 2-3 days.

Quality control

To quality-control formed spheroids, we recommend performing a minimal set of analyses after aggregation:

1. Morphological assessment:

During aggregation, regular spherical tissue structures with distinct edges form. Assess aggregated tissues under an inverted microscope and compare morphologies to provided reference images and plate scans (Figure 4).

2. Size determination:

Determination of spheroid diameters using software allowing image-based object measurements (e.g., ImageJ, Download (nih.gov)). The 1000 µm well bottom can be used as a calibrator. Expected tissue diameters are approximately 250 µm for 1150 cells seeded per well.

3. ATP content determination:

ATP content measurements of formed tissues on day 5 after aggregation. The respective protocol for ATP content measurements using the CellTiter-Glo reagent are available on our webpage (TP001). Expected ATP contents should be > 10 pmol ATP/spheroid for 1150 cells seeded per well.

References

Bell CC, Dankers ACA, Lauschke VM, et al. Comparison of Hepatic 2D Sandwich Cultures and 3D Spheroids for Long-term Toxicity Applications: A Multicenter Study. *Toxicol Sci.* 2018;162(2):655-666. doi:10.1093/toxsci/kfx289

Messner S, Agarkova I, Moritz W, Kelm JM. Multi-cell type human liver microtissues for hepatotoxicity testing. *Arch Toxicol.* 2013;87(1):209-213. doi:10.1007/s00204-012-0968-2

Proctor WR, Foster AJ, Vogt J, et al. Utility of spherical human liver microtissues for prediction of clinical drug-induced liver injury. *Arch Toxicol.* 2017;91(8):2849-2863. doi:10.1007/s00204-017-2002-1

Schulze RJ, Schott MB, Casey CA, Tuma PL, McNiven MA. The cell biology of the hepatocyte: A membrane trafficking machine. *J Cell Biol.* 2019;218(7):2096-2112. doi:10.1083/jcb.201903090

Appendix A

Technical Protocols

Table 2:

Complementary protocols

Name	Number	Description	Link
Microtissue CTG 3D	TP001	Determination of ATP content in Spheroids	Link
Whole mount microtissue staining	TP008	Preparation of Spheroids for whole mount stainings	Link

Spheroid collection

The special coating of the Akura™ Plate minimizes the adherence of the spheroids to the bottom of the well. This facilitates collection of spheroids for transfer into another plate format or for further processing, such as embedding for histological analysis. To harvest spheroids, we recommend the following protocol:

1. Gently immerse a pipette, holding a 1000 µl tip, along the inside wall of the well, until feeling a slight resistance. The pipette tip is now positioned slightly above the spheroid on the well bottom (Figure 6A). Use of 1000 µl tips prevents the spheroid from being squeezed inadvertently because the tip diameter exceeds the size of the well bottom.

OR

Alternatively, use a 100–200 µl tip and carefully lower the tip at a slightly angled position along the wall until it touches the well bottom. Aspirate by placing the head of the tip close to the edge of the well bottom (Figure 6B). Note that incorrect positioning of the 100–200 µl pipette may damage spheroids (Figure 6C).

2. Collect the spheroid by aspirating 50 µl of the medium. Avoid aspiration of air bubbles to prevent spheroid loss in the pipette tip.
3. Transfer the spheroid in medium into another vessel or plate.

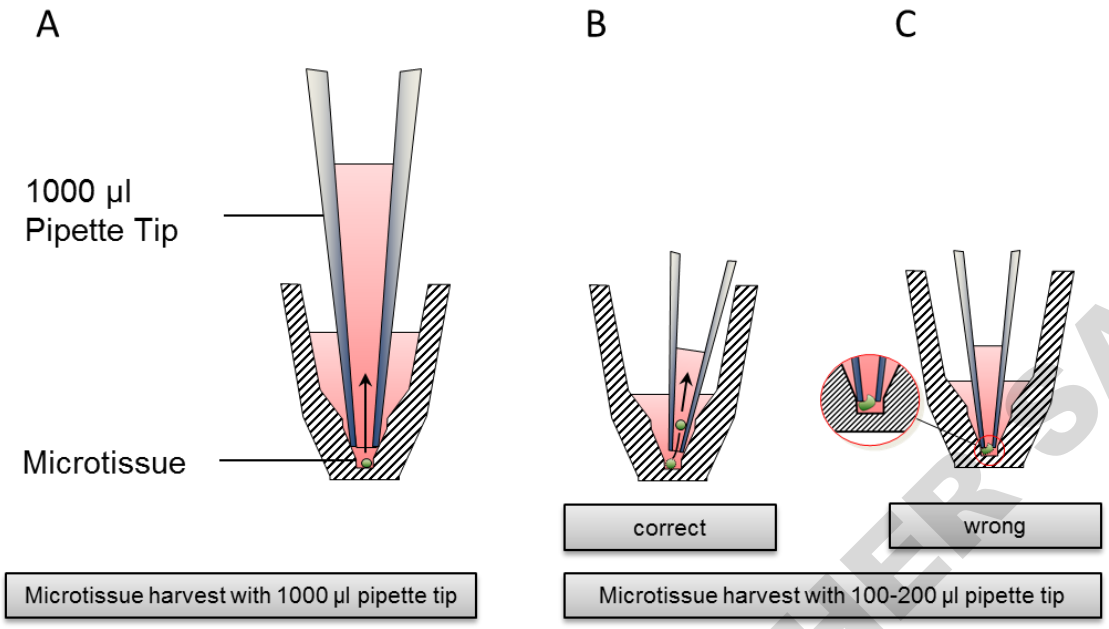


Figure 6: Pipette positioning when collecting spheroids using A. a 1000 µl pipette tip or B. a 200 µl pipette tip. C. The incorrect way to position a 200 µl pipette tip during transfer, causing spheroid damage.

Compound dosing & re-dosing

Compound Preparation

To adjust 0.5 % DMSO (v/v) final concentration in culture medium, prepare a 200X top compound concentration stock in DMSO.

- Prepare 6 dilutions of the compound stock in DMSO using a sterile V-bottom microplate. Choose the dilution factor depending on the range of concentrations to be tested in the assay[5].
- Transfer 2.5 µl of each compound dilution to the corresponding well on a sterile deep well plate as presented in Figure 7 (upper panel). For each planned re-dosing prepare a separate deep well plate[6]
- For vehicle controls, pipet 2.5 µl of DMSO to column 4 on a deep well plate.
- Seal deep well plates and store at –20°C for future re-dosing.

Dosing

- Scan plates or examine under an inverted microscope before compound exposure. Exchange individual spheroids if needed.
- Thaw deep well plates with compound(s) to be tested and add to each experimental well 497.5 µl of pre-warmed maintenance medium, thereby generating 1 X top concentration of the compound and its corresponding dilutions with 0.5 % DMSO (v/v).
- Gently aspirate culture medium from the Akura 96™ Plate, leaving spheroids in the remnant volume of the medium in the well.

- Thoroughly mix medium with compound in the deep well plate and dose 70 µl per spheroid in required number of replicates (Figure 7, bottom).
- To control the DMSO effect on spheroids, add 70 µl of culture medium per well to column 3 on the Akura™ 96 Plates (Figure 7, bottom).
- Repeat dosing at required time intervals.

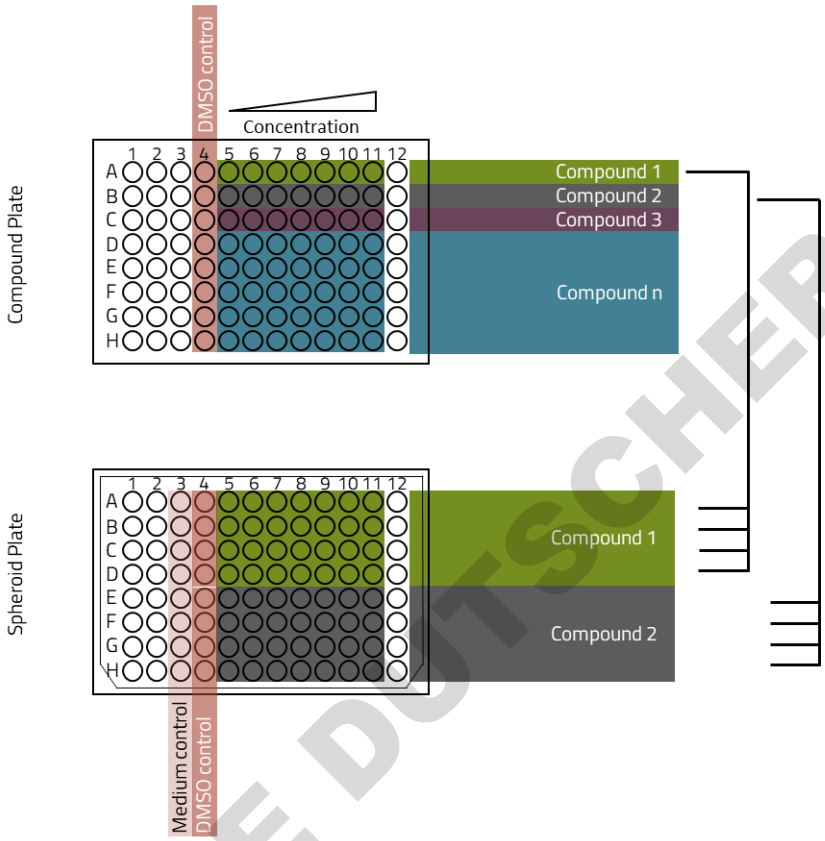


Figure 7. Schematic overview of compound plate preparation and treatment scheme.

[5] *To generate reference data a top concentration of 100 µM and 6 stepwise half-logarithmic dilutions were used.*

[6] *To generate reference data, one re-dosing at day 4 was performed for 7-day treatments and two re-dosings at day 5 and day 9 were performed for 14-day treatments.*

Appendix B: Donor Information

Table 3

Serology of donor material

Gender	**Male**
Age	26 years of age
Race	Caucasian
Cause of Death	Head Trauma
Antibody to CMV	Negative
HIV	Negative
Hepatitis B Surface Antigen (HBsAg)	Negative
Hepatitis C Virus	Negative
Rapid Plasma Reagin	Negative

CAUTION:

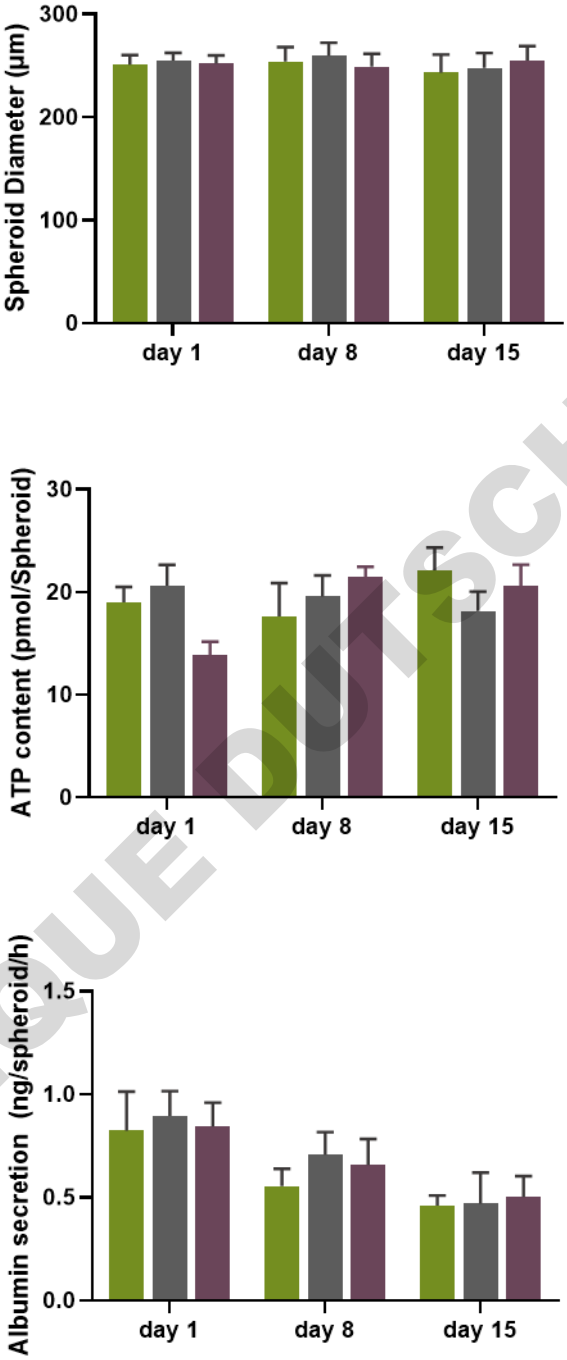
This sample should be considered as a potential biohazard and universal precautions should be followed. Intended for in vitro use only.

Table 4

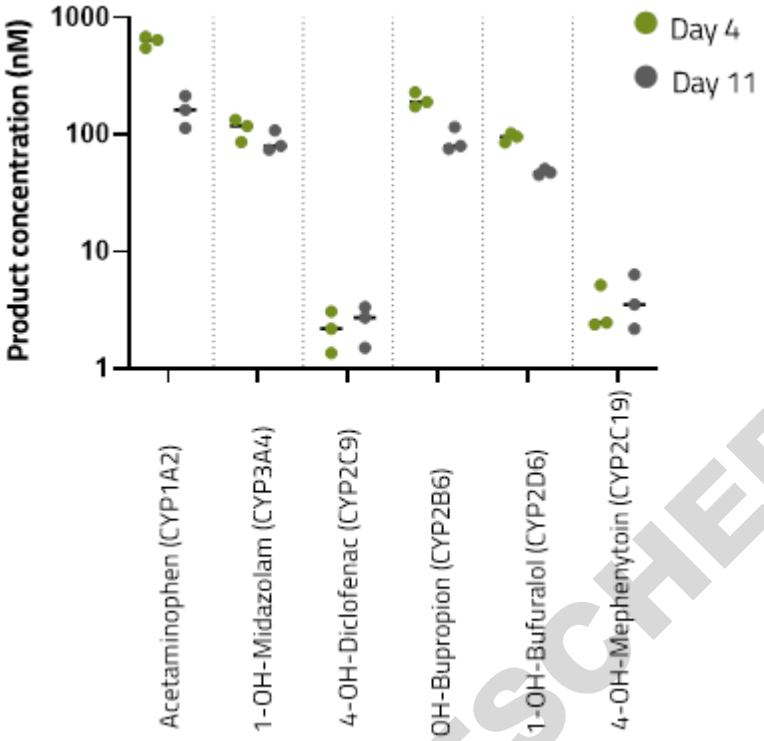
Cytochrome activities: Values for enzyme activities were determined at a single substrate concentration and are mean + standard deviation of three or more determinations.

Enzyme	**Marker Substrate Reaction**	**[S] (µM)**	**Rate (pmol/million cells/min)**
CYP1A2	Phenacetin *O*-dealkylation	100	12.2 ± 1.5
CYP2A6	Coumarin 7-hydroxylation	50	5.17 ± 0.56
CYP2B6	Bupropion hydroxylation	500	8.60 ± 0.66
CYP2C8	Amodiaquine *N*-dealkylation	20	52.7 ± 7.6
CYP2C9	Diclofenac 4'-hydroxylation	100	63.2 ± 0.7
CYP2C19	*S*-Mephenytoin 4'-hydroxylation	400	6.24 ± 0.36
CYP2D6	Dextromethorphan *O*-demethylation	80	49.8 ± 3.9
CYP2E1	Chlorzoxazone 6-hydroxylation	500	65.5 ± 3.5
CYP3A4/5	Testosterone 6β-hydroxylation	250	52.6 ± 4.9
CYP3A4/5	Midazolam 1'-hydroxylation	30	6.09 ± 0.74

Appendix C: Characterization Data



Characterization Data of hepatocyte spheroids (1150 cells/spheroid) generated with the Akura™ 3D SureKit. Size stability and homogeneity of tissues (top), Spheroid viability by ATP content (middle) and tissue functionality by albumin secretion (bottom) were assessed over the course of three independent aggregation runs (■run 1, ■run 2, ■run 3).



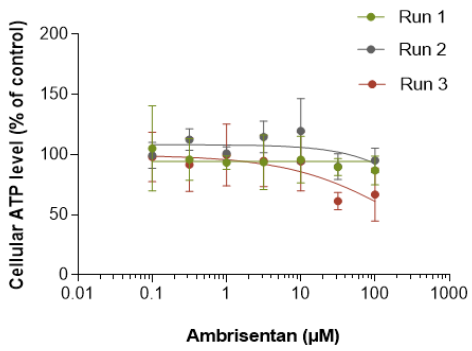
Cytochrome induction data for Akura™ 3D SureKit aggregated hepatocyte spheroids (1150 hepatocytes/spheroid). Product formation for 6 Cyp iso-form specific educts was quantified at two experimental timepoints by LC-MS.

Appendix D: Reference Compound Treatments

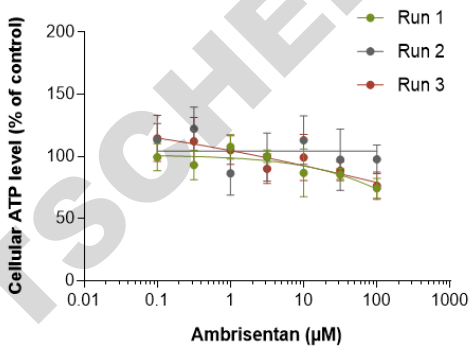
Ambrisentan

	Mean IC_{50}	Margin of safety (IC_{50}/C_{max})
7-day exposure Akura™ 3D SureKit	>100	>100
14-day exposure Akura™ 3D SureKit	>100	>100

7-day exposure



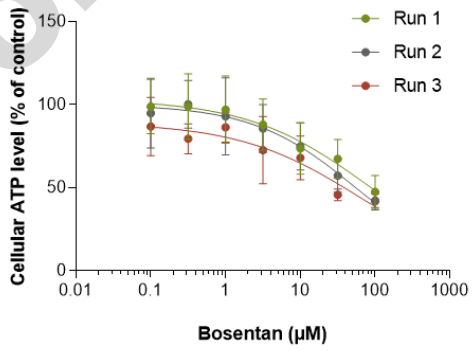
14-day exposure



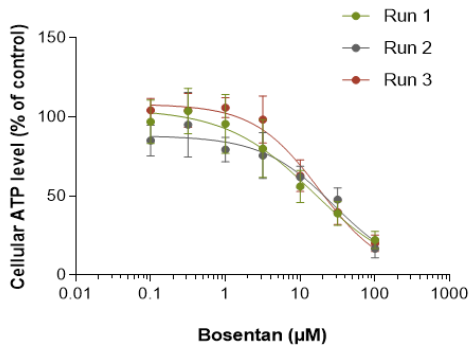
Bosentan

	Mean IC_{50}	Margin of safety (IC_{50}/C_{max})
7-day exposure Akura™ 3D SureKit	65.0	8.7
14-day exposure Akura™ 3D SureKit	20.4	2.7

7-day exposure



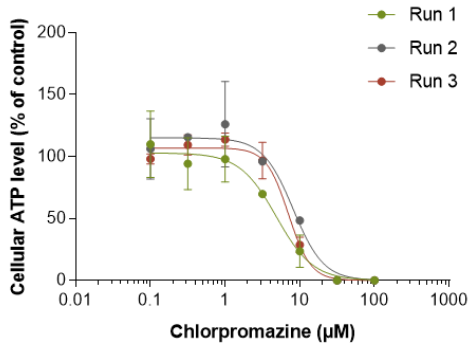
14-day exposure



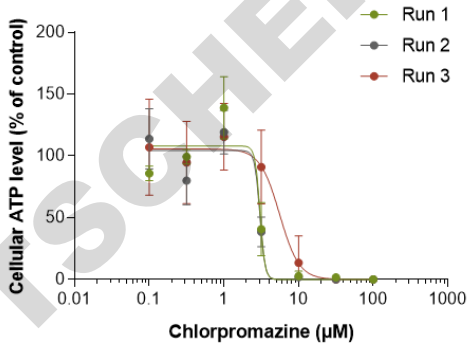
Chlorpromazine

	Mean IC_{50}	Margin of safety (IC_{50}/C_{max})
7-day exposure Akura™ 3D SureKit	6.7	7.1
14-day exposure Akura™ 3D SureKit	3.9	4.1

7-day exposure



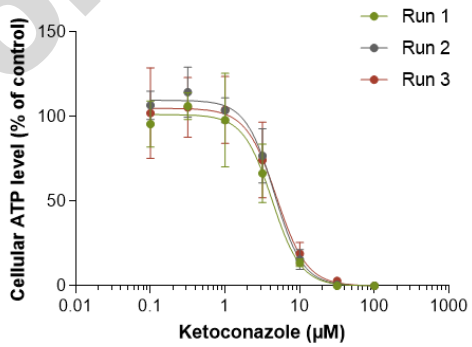
14-day exposure



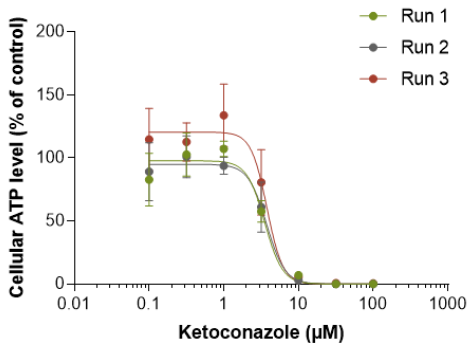
Ketoconazole

	Mean IC_{50}	Margin of safety (IC_{50}/C_{max})
7-day exposure Akura™ 3D SureKit	4.6	0.4
14-day exposure Akura™ 3D SureKit	3.7	0.3

7-day exposure



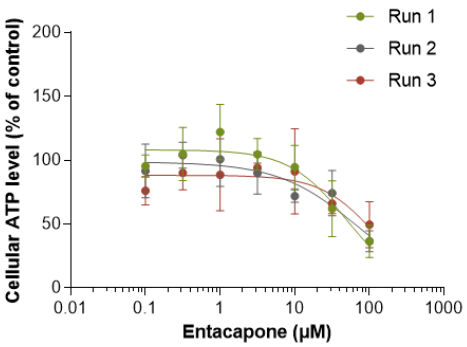
14-day exposure



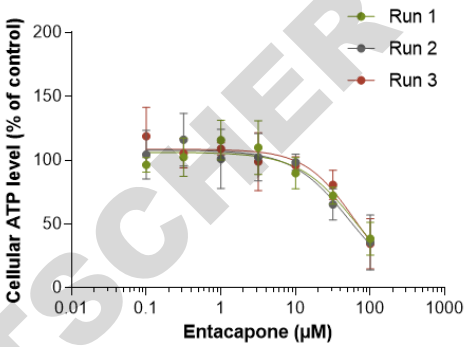
Entacapone

	Mean IC_{50}	Margin of safety (IC_{50}/C_{max})
7-day exposure Akura™ 3D SureKit	75.8	23.1
14-day exposure Akura™ 3D SureKit	57.4	17.5

7-day exposure



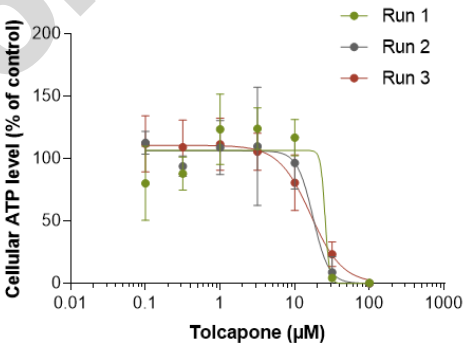
14-day exposure



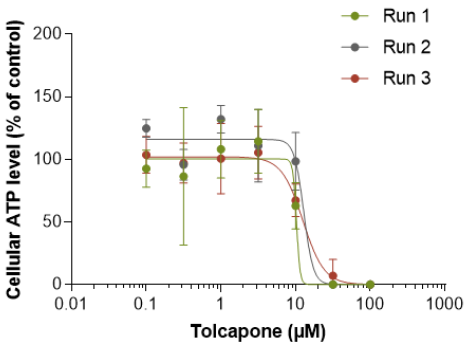
Tolcapone

	Mean IC_{50}	Margin of safety (IC_{50}/C_{max})
7-day exposure Akura™ 3D SureKit	17.4	0.4
14-day exposure Akura™ 3D SureKit	11.5	0.2

7-day exposure

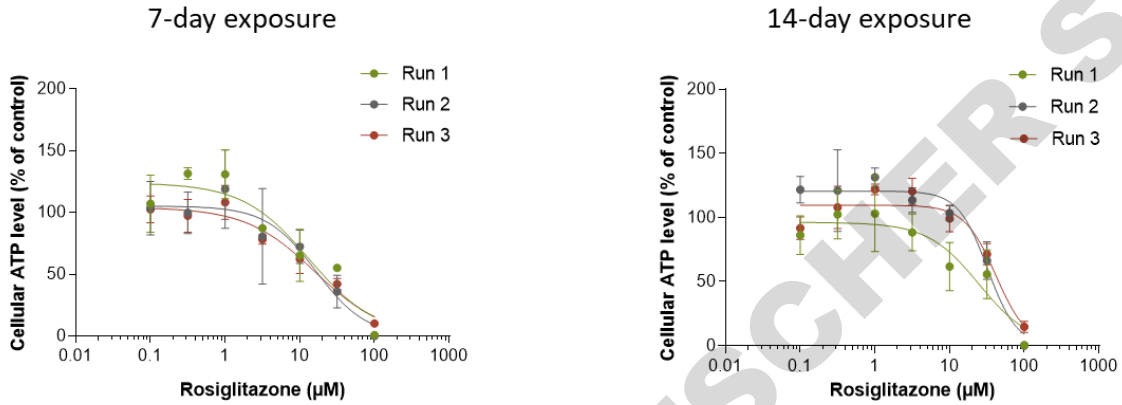


14-day exposure



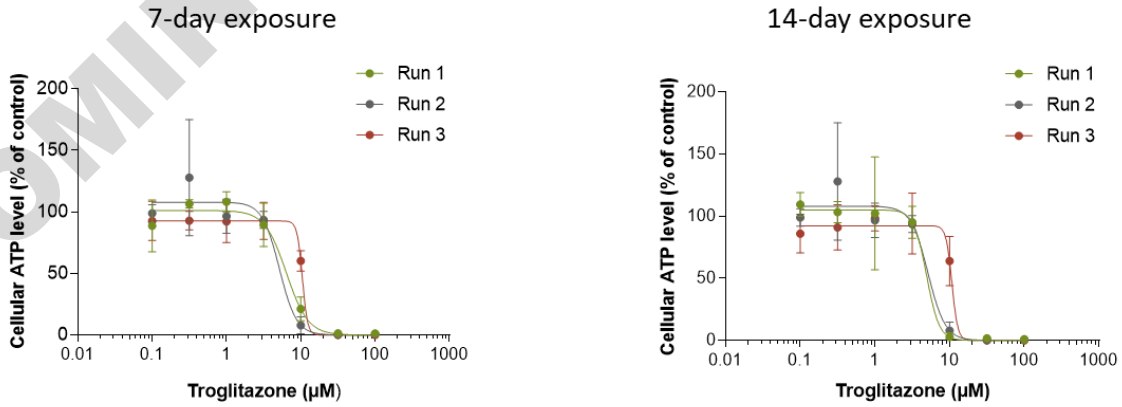
Rosiglitazone

	Mean IC_{50}	Margin of safety (IC_{50}/C_{max})
7-day exposure Akura™ 3D SureKit	20.9	20.9
14-day exposure Akura™ 3D SureKit	32.8	32.8



Troglitazone

	Mean IC_{50}	Margin of safety (IC_{50}/C_{max})
7-day exposure Akura™ 3D SureKit	7.6	1.2
14-day exposure Akura™ 3D SureKit	8.0	1.3



Dose-response curves for five test substances using Akura™ 3D SureKit aggregated hepatocyte spheroids (C_{max} values see Table 5)

Table 5

DILI compounds used to characterize Akura™ 3D SureKit aggregated human liver spheroids

Compound	Clinical DILI	Description	DILI pattern	Status/Label	C_{Max}* (µM)	DILI classification
Ambrisentan (CAS: 177036-94-1)	No	Endothelin receptor antagonist	n/a	Boxed warning	0.8	No DILI
Bosentan (CAS: 147536-97-8)	Yes	Endothelin receptor antagonist	Cholestatic	Black box warning	7.4	Severe clinical DILI
Chlorpromazine (CAS: 50-53-3)	Yes	Antipsychotic agent.	Cholestatic	Adverse reactions	0.9	Low clinical DILI concern
Ketoconazole (CAS: 65277-42-1)	Yes	Anti fungal	Mixed	Black box warning	11.3	Severe clinical DILI
Entacapone (CAS: 130929-57-6)	No	Catechol-O-methyl-transferase inhibitor	Enzyme elevations	No warning	3.3	Enzyme elevation in clinic
Tolcapone (CAS: 134308-13-7)	Yes	Catechol-O-methyl-transferase inhibitor	Hepatocellular	Black box warning	47.6	Severe clinical DILI
Rosiglitazone (CAS: 122320-73-4)	Yes	Antidiabetic	Mixed	Warnings and precautions	1,0	High clinical DILI concern
Troglitazone (CAS: 97322-87-7)	Yes	Antidiabetic	Mixed	Withdrawn	6.4	Severe Clinical DILI

* Proctor WR, Foster AJ, Vogt J, et al. Utility of spherical human liver microtissues for prediction of clinical drug-induced liver injury. *Arch Toxicol*. 2017;91(8):2849-2863. doi:10.1007/s00204-017-2002-1

Appendix E: Akura™ 96 Plate Specifications

Product Description:

The Akura™ 96 Spheroid Microplate format is compliant with standard microtiter-plate definitions as specified by the SLAS Microplate Standards Advisory Committee ANSI SLAS 1-2004 (R2012). The 96 wells are arranged in 8 rows and 12 columns, identified by alphanumeric labels (Figure 8A). Individual wells show a regular wide opening at the top narrowing down into a small cavity at the well bottom, with a flat optically clear base (Figure 8B), designed to accommodate spheroids of up to 750 µm in diameter. The Akura™ 96 Spheroid Microplate technical specifications are provided as a reference for automation system programming (Figure 9).

A

B

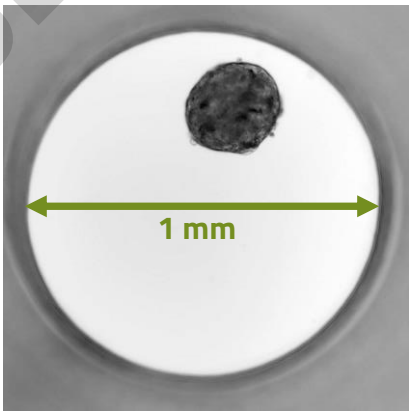


Figure 8: A. Angled view of Akura™ 96 Plate. B. Human liver microtissue in Akura™ 96 Plate. The well diameter is exactly 1 mm.

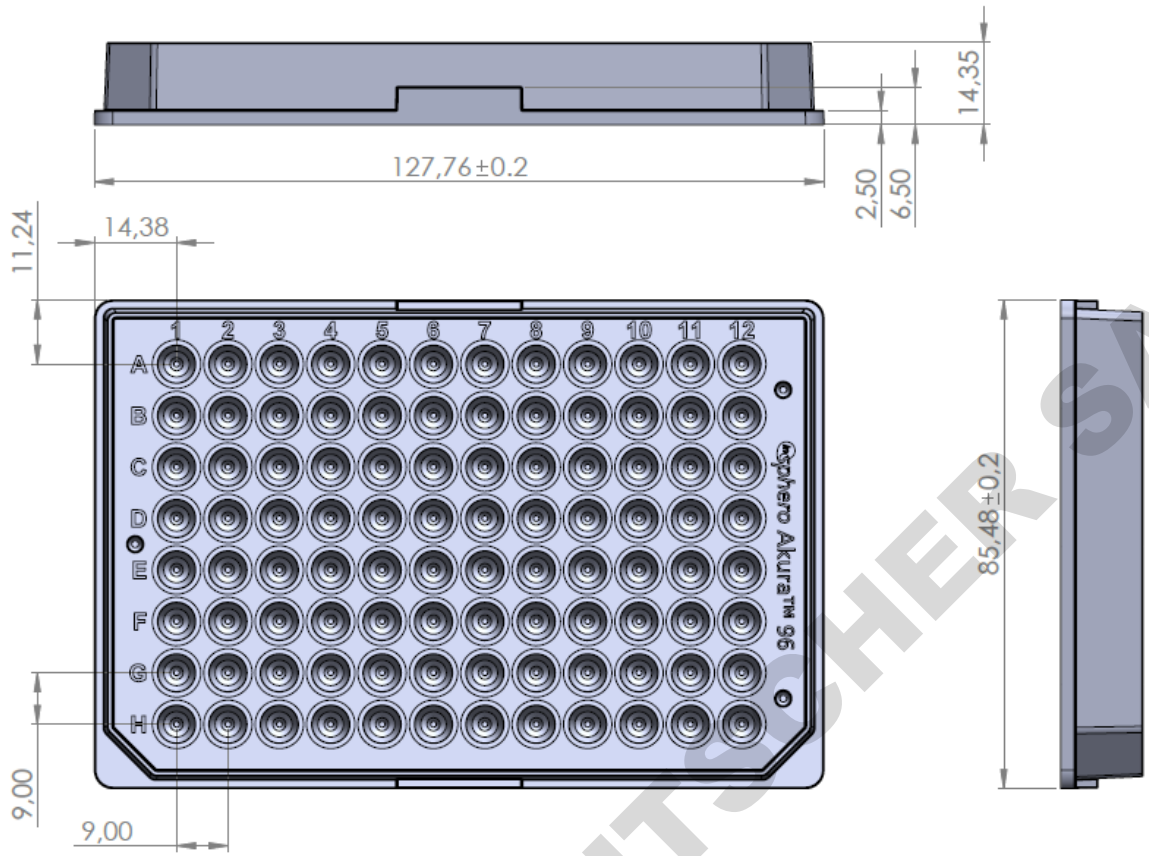


Figure 9: Technical specifications of Akura™ 96 Plate in mm.

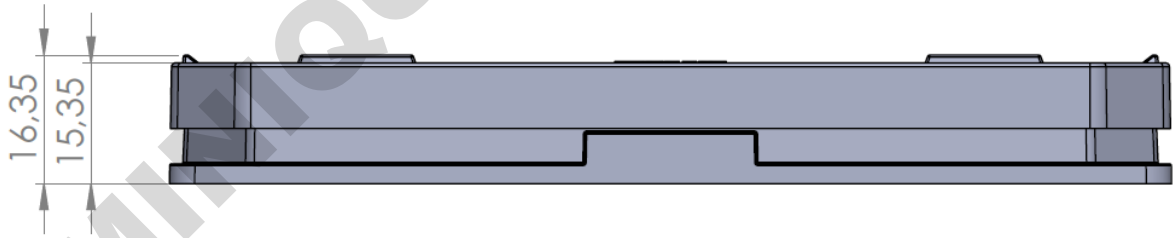


Figure 10: Height of well with lid in mm.

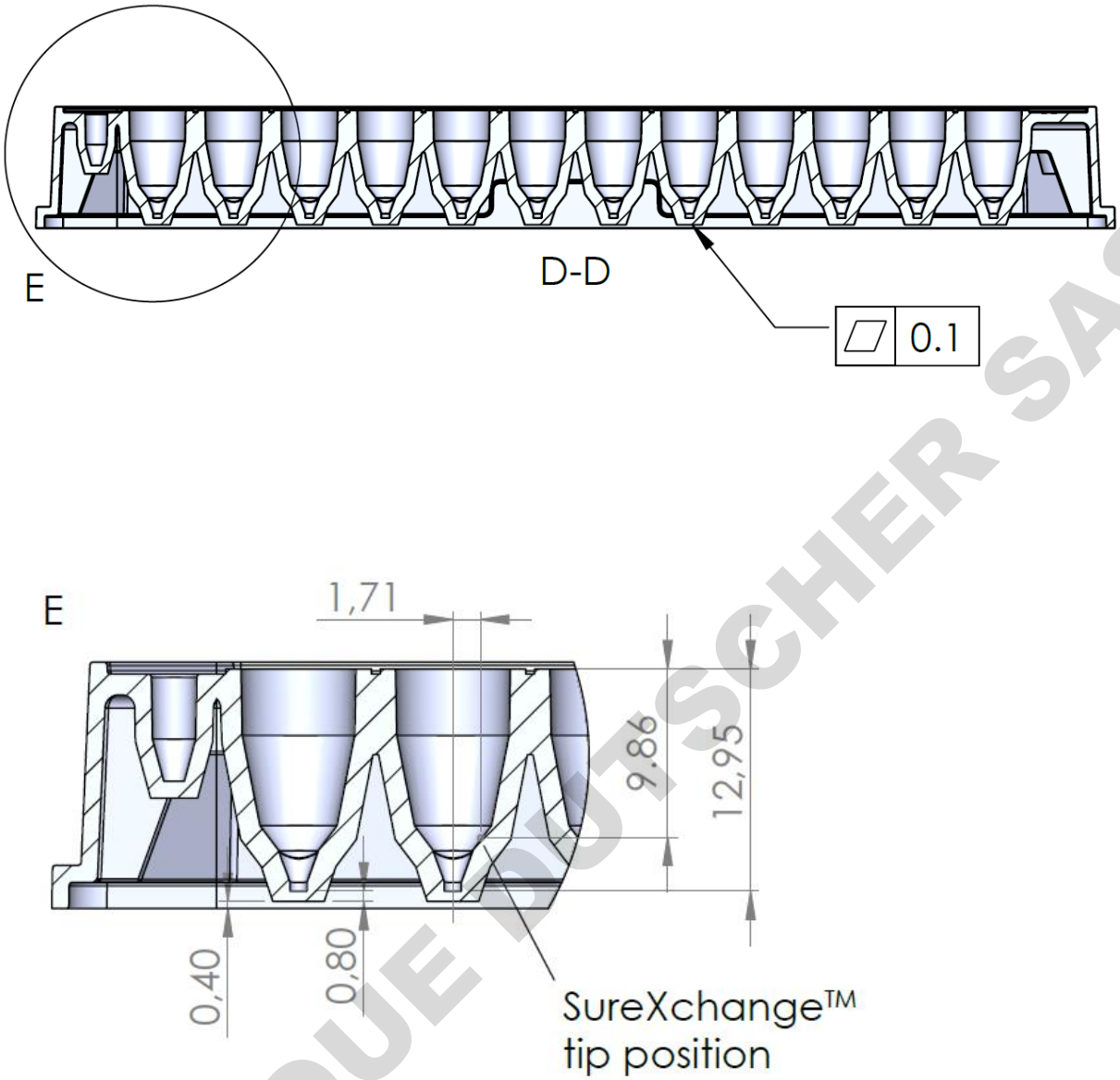


Figure 11: Height of well, skirt height, well bottom thickness and SureXchange™ tip position in mm.

Appendix F: Akura™ Tilting Stand

The Akura™ Tilting Stand is designed to hold up 1 to 4 Akura™ Spheroid Microplates at a defined angle for directing seeded cells to the edge of the well and enable more efficient and controlled spheroid aggregation (Figure 12).

Microplates are placed on the plate area with the long-side facing down. The Akura™ Tilting Stand can be sterilized using conventional methods.

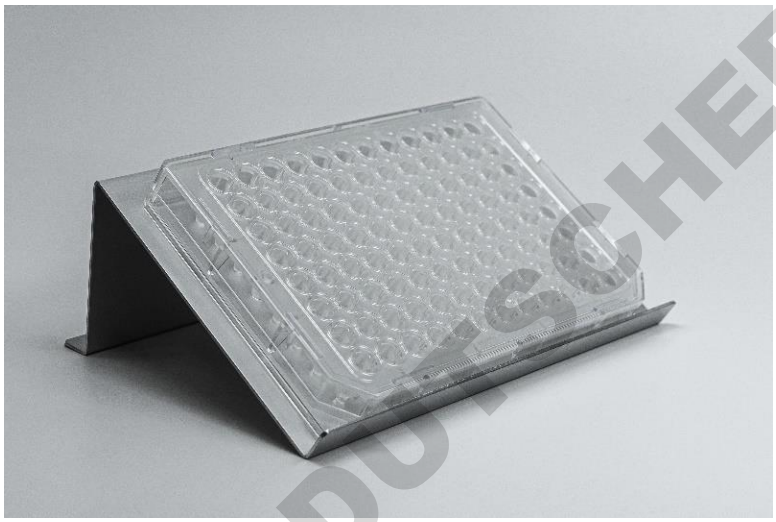


Figure 12: Akura™ Tilting Stand with one Akura™ 96 Spheroid Microplate.

Appendix G: Frequently Asked Questions

A detailed protocol for production of hepatocyte spheroids is provided in this product manual. Below are answers to some frequently asked questions to help get you started.

Q: What is the optimal volume per well in the Akura™ 96 Plate?

A: To achieve optimal volume per well, gently deliver 70 µl (pipetting speed < 10 µl/sec) of cell suspension into each well of the Akura™ 96 Plate by placing the pipette tips near, but not touching, the bottom of the wells.

Important - For spheroids with uniform size and cell composition it is essential to assure a homogeneous distribution of the cells by gently pipetting up and down prior to seeding into the Akura™ 96 Plate.

Q: Why do you recommend centrifuging the Akura™ 96 Plate after cell seeding?

A: We recommend to briefly centrifuge the plate after cell seeding to remove any air bubbles and to force the cells to the bottom of the well in order to promote cell-aggregation and spheroid formation.

For that, place the lid on the plate and spin in a microtiter-plate centrifuge for 2 minutes at 200 RCF. Afterwards, incubate the plate in a humidified CO^2 incubator at 37 °C for 2-5 days.

Q: Are there enough cells in the vial to aggregate larger tissues?

A: Yes, the vial contains sufficient cell numbers to aggregate large spheroids (up to 2500 cells/spheroid)

Q: How do I exchange the medium in the Akura™ 96 Plate without disturbing or losing the spheroids?

A: To prevent spheroid/organoid loss during the exchange of media, the SureXchange™ ledge serves as an anchoring point for the pipette tip. Just place the tip at the ledge of the well, see figure below, and remove the medium at low pipetting speed (>30 µl/sec). A minimal volume of ~ 5-7 µl will remain in the well.

Then, add 70 µl of fresh medium by placing the pipette tip at the ledge, use dispensing rate <50 µl/sec.

Important - when using automated liquid handling devices, an off-center alignment of the vertical pipette tip will achieve the same effect.

Q: What is the best way to prevent evaporation in the outer wells of my plates?

A: Evaporation in the outer (perimeter) rows of wells is a phenomenon common to most low-volume culture platforms, and thus requires careful attention to maintaining proper humidity control. If not controlled, pronounced evaporation can result in concentration or precipitation of media components (serum, salt) that can impact spheroid formation or health, and can alter the effective concentration of a compound/additive in the medium over the course of a long-term experiment. To provide maximum humidity control when using the Akura™ Plates, we recommend the following:

Use an incubator with good humidity control (>95% of rel. humidity), and exercise best practice in maintaining and minimizing loss of humidity (e.g., minimize incubator door opening and closing).

For culture in the Akura™ 96 Plate, at least 50-70 µl of medium in each well is recommended and can be increased to a maximum of 80 µl if incubator humidity control is a persistent issue. Medium exchange frequency can also be increased to every other day or daily if conditions dictate.

We recommend the use of the InSphero Incubox™ to reduce edge effects when performing long-term culture with low-frequency medium exchange. The Incubox™ is available in shop.insphero.com.

InSphero AG
Schlieren, Switzerland

InSphero Inc.
Brunswick, ME, USA

If you have more questions, please refer to the FAQs section on shop.insphero.com

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