

revvity

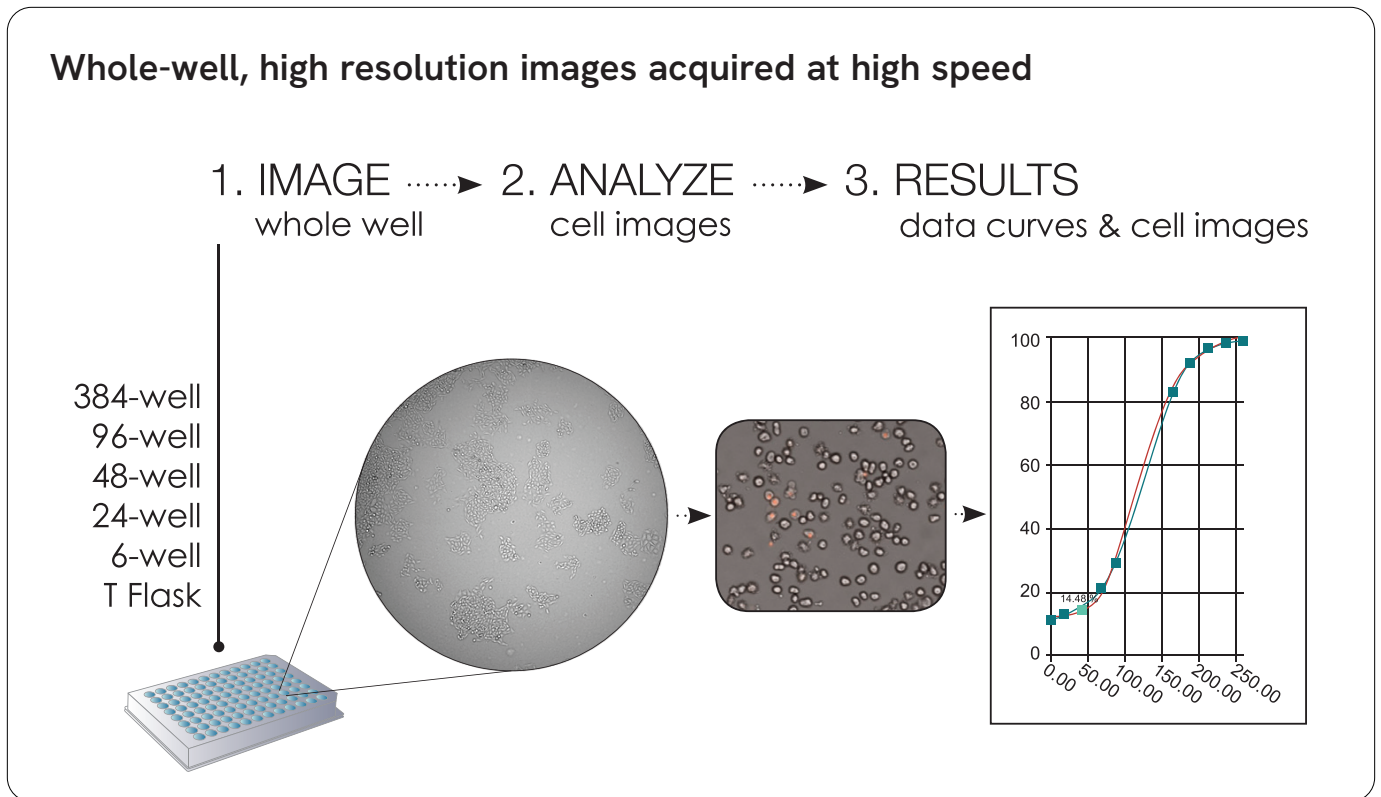
# Characterizing hepatocytes for high quality ADME assays.

Celigo® Image Cytometer

Cell Count ▪ Viability ▪ Apoptosis



## Introduction to the Celigo image cytometer



### Image

- Proprietary optical design enables uniform illumination and consistent edge contrast
- Image and count every cell in each well: 0 - 100,000 cells/96-well
- 5 imaging channels with brightfield and 4 fluorescent channels

### Analyze

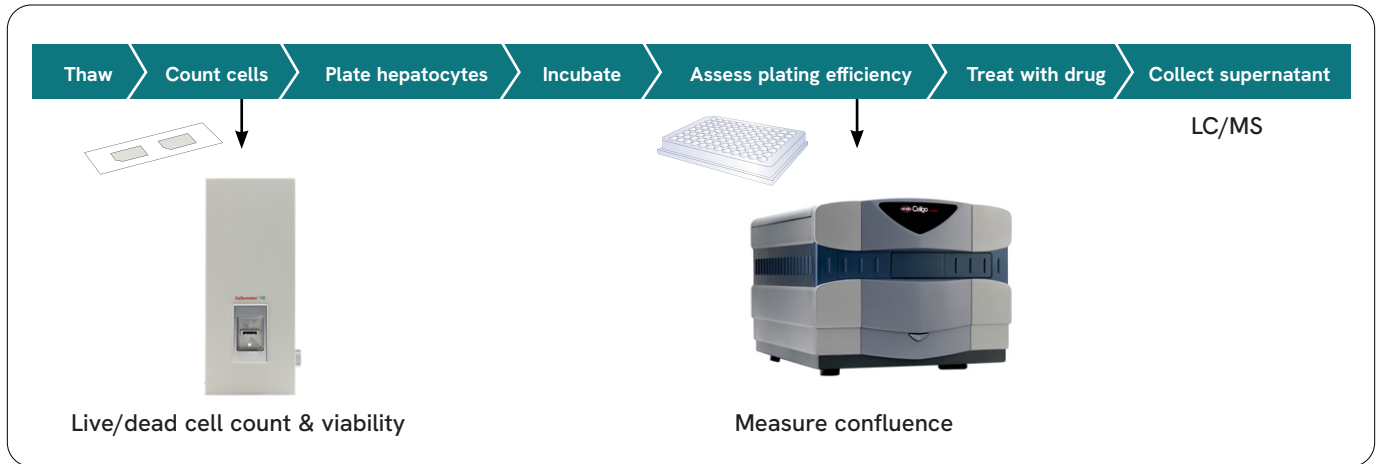
- Fast scanning for image acquisition and analysis with minimal plate movement ensuring minimal sample disruption
- Accurately quantify cells and colonies with a non-invasive method
- Measure adherent cells without trypsinization

### Results

- Save experiment settings - quickly run the same assay on many plates without additional set up
- System automatically stitches multiple fields of view into a single full resolution image

## Improve hepatocyte characterization for ADME/PK assays

ADME/PK assays rely on accurate seeding density, hepatocyte viability, plateability/adherence and intact hepatobiliary transport. Revvity provides the Celigo imaging system, reagents and quantification software to characterize primary hepatocytes in 2D, 3D and co-culture models.



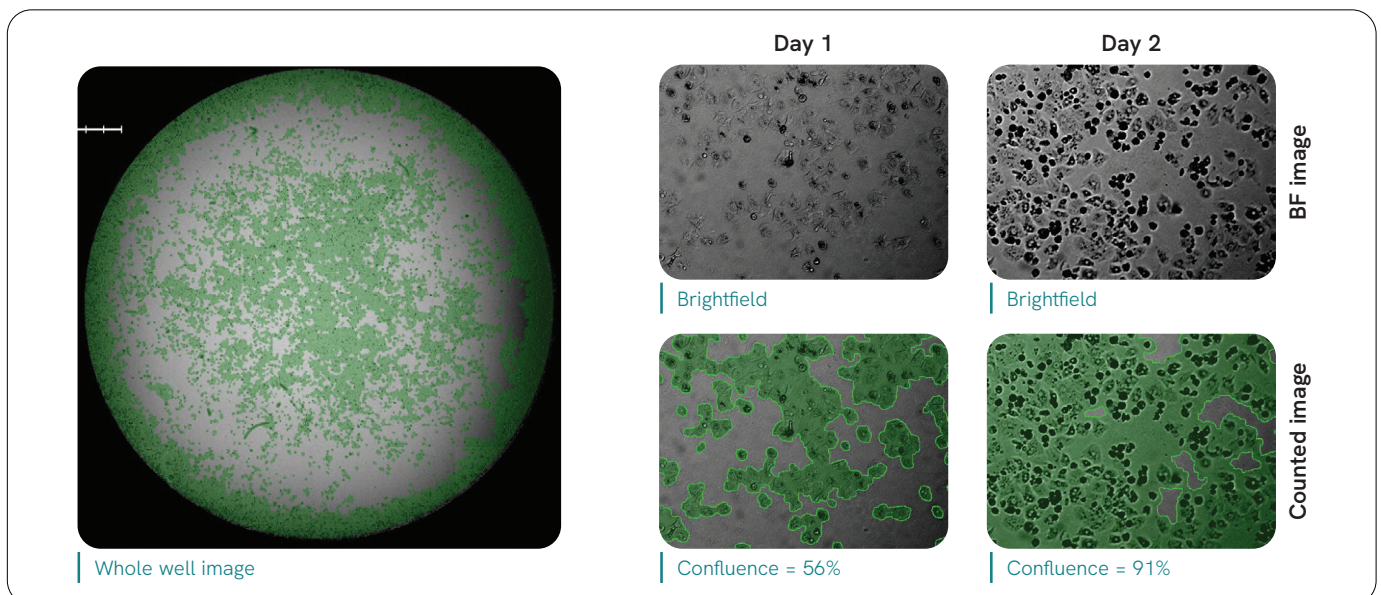
### 1. Monitor plated hepatocytes using confluence in a 96-well plate

#### Simplify workflow & protect your cells

Celigo's label free confluence application provides a quick tool to visualize cell morphology and assess growth. A single plate can be scanned and imaged for an entire week without having to trypsinize cells.

#### Troubleshoot & rank plateability

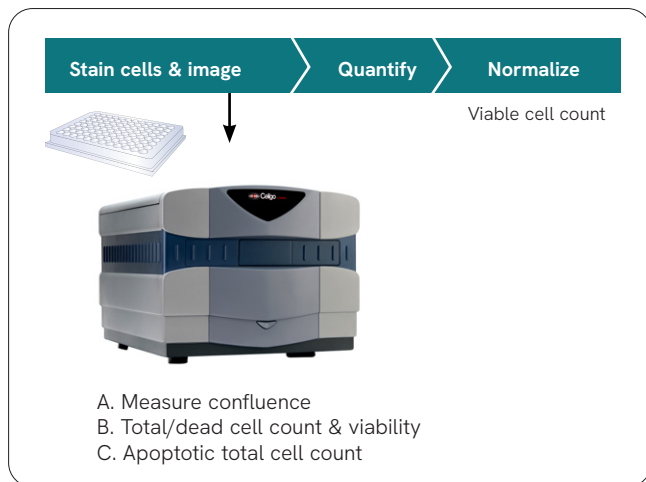
Type, source, seeding density and time affect the maintenance of healthy monolayers for plateable hepatocytes. Celigo provides a non-invasive method for ranking plateability and generates comprehensive growth tracking reports for plateable hepatocytes.



## 2. Measure cell count, viability or apoptosis of plated primary hepatocytes in a 96-well plate

### Accurately segment & count adherent hepatocytes

Celigo algorithms can be customized to accurately count single or poly-nucleated hepatocytes. Celigo analyzes fluorescence channels (Hoechst+) to provide accurate and consistent cell counts for plateable hepatocytes.



### Multiplex assays for hepatocyte health

#### Viability assay using DNA binding dye [Hoechst/PI]

As an end point assay, Celigo gives quick readouts for percent viability using Hoechst (blue, DNA binding dye) & PI (red, dead dye).

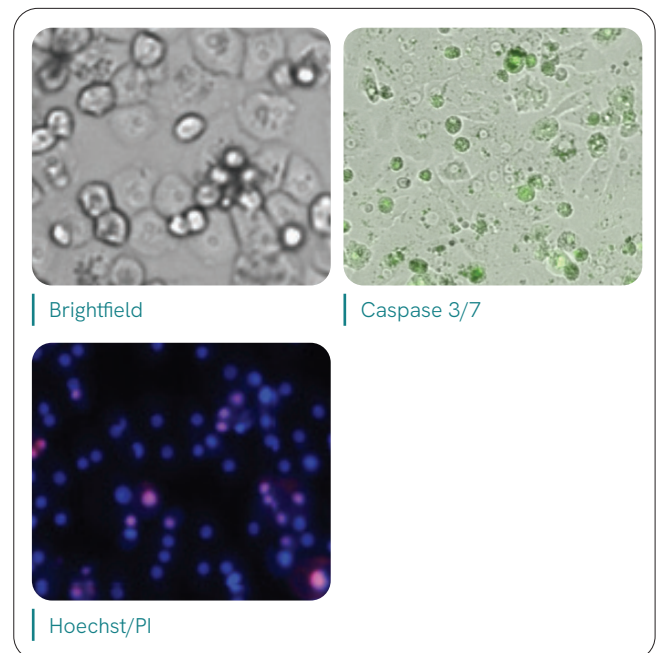
#### Early apoptosis by measuring [Caspase 3/7]

If Caspase 3/7 is expressed in the cell, the cell will fluoresce green. Activation of Caspase 3/7 can be monitored as an end-point assay using fluorescent (FL) expression suite of applications on the Celigo. Values for % apoptosis can be normalized to cell counts from the brightfield or blue channel (Hoechst+).

Stain	Assay Output
Hoechst / PI	Total cell number Dead cell number Viability
Hoechst / Caspase 3/7	Total cell number Total apoptotic cell number Percent apoptotic cells

### Optional: kinetically monitor apoptosis using [Caspase 3/7]

Activation of Caspase 3/7 can also be monitored as real-time using FL expression suite of applications on the Celigo.



**“We were able to scan 85 plates in five days. Celigo was easy to use and very fast. It captured images of primary hepatocytes in 12-, 24- and 96-well plate formats. Without the Celigo, it would have taken us over a month to complete the hepatocyte characterization reports for this scope of work.**

### 3. High-Throughput Cell Concentration & Viability for Suspension Hepatocytes in 96-Well Plates

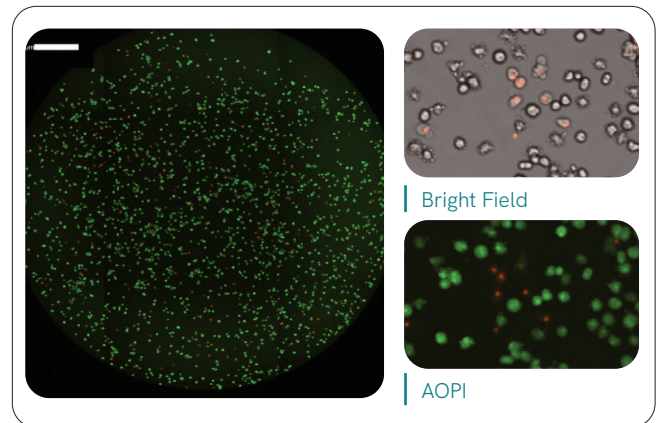
#### Characterization for suspension hepatocytes

- Live/dead cell count
- Viability
- Satin with AO/PI
- Image in brightfield, green and red channels

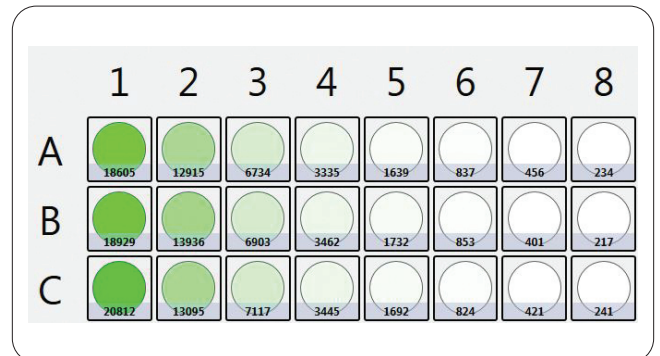
Celigo images accurately segment and count every cell in every well with minimal plate movement and minimal sample disruption.

#### User-friendly data management & workflow

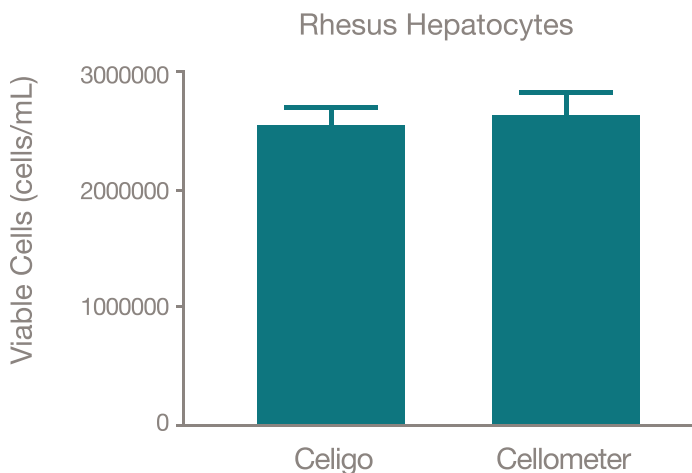
Celigo image acquisition and analysis occurs simultaneously in one easy workflow to accommodate multiple users. High resolution images and experimental settings can be saved and easily accessed by each user. With the Celigo, counting can be done for adherent or suspension hepatocytes in 12, 24 or 96 well plates.



Whole well image view of hepatocytes in suspension after staining with AO (green, live dye) & PI (red, dead dye). Celigo captures and analyzes cells in brightfield, green and red channels.



An automatic heat map of live hepatocytes (in suspension) is generated for the entire experiment providing an easy visual overview of the number of cells present in each well. The results from the Celigo plate view shows consistent cell counts (performed in triplicate) with a broad dynamic range of cell concentrations (20,812 - 217 cells/well).



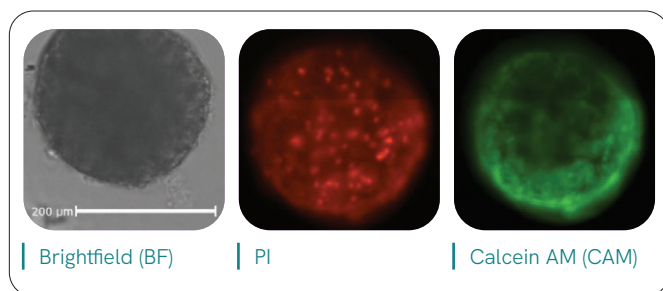
#### Improve throughput without sacrificing quality

Celigo and Hepatometer (Cellometer) viability data is complementary to each other. Hepatometer (Cellometer) is a widely used fluorescent based cell counter that calculates cell concentration and viability of primary cells in suspension using the live/dead dye, AOPI. Counting on a Cellometer takes 60 seconds per sample. Celigo can image and analyze a 96-well plate stained with AOPI in less than 10 minutes.

## 4. Image and quantify 3D spheroids for size, viability and apoptosis in U-bottom plates

### Monitor spheroid size & viability

Celigo 3D applications are very fast. Tumorsphere size application in brightfield takes 2 minutes for an entire 96-well plate. A tumorsphere viability application is done in 7 minutes for 96-well plate.



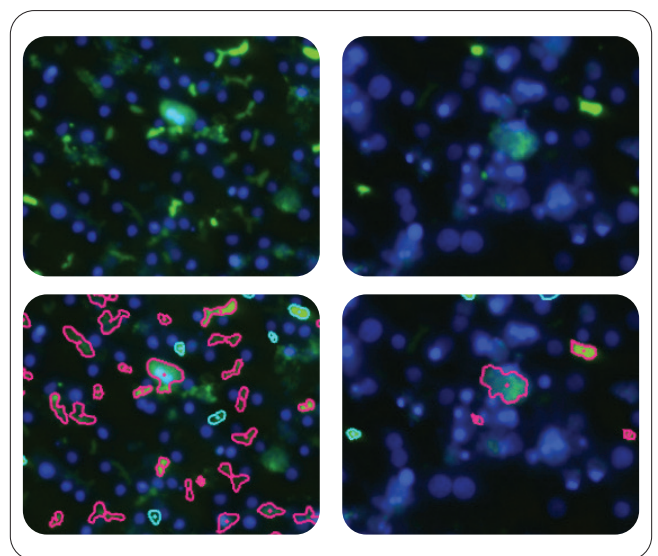
Celigo images of stem cell derived tumor spheroids treated with 2% DMSO & stained with live green dye (Calcein AM), and dead cell red dye (PI) to examine viability.

Immune-mediated drug-induced hepatotoxicity is often unrecognized as a potential mode of action due to the lack of appropriate *in vitro* models. With the Celigo, you can widen the scope of research to include cocultures of stromal cells, immune cells and primary hepatocytes.

Citation: Rose KA, Holman NS, Green AM, Andersen ME, LeCluyse EL3 *J Pharm Sci.* 2016 Feb;105(2):950-64. doi: 10.1016/S0022-3549(15)00192-6.

### Bile-canaliculi formation as a measure of transport capacity in co-culture models

Celigo can be used to count the number and analyze the shape and structure of bile canaliculi (BC) in a single or co-culture of hepatocytes. Presence of intact, 3D or tube-like BC is an indicator of intact transport capacity. Bile canaliculi are labeled with a MRP2 fluorescent substrate, CDF, which fluoresces green when in its non-fluorescent form (CDFDA) is cleaved by intracellular esterases.



“Thanks to the Celigo, we are now performing and monitoring 3D tumor spheroid growth inhibition routinely and easily. The multiplexing capacities of the machine are used regularly for organelle visualization, apoptosis and cell cycle assays; which highly decreases our use of a standard flow cytometer and increases our throughput by using mostly 96- and 384-well plates. Overall, the Celigo is very user friendly and we are very happy with our acquisition.

## Hepatocyte characterization report on Celigo

### Set a new gold standard with the celigo

Celigo 3D applications are very fast. Tumorsphere size application in brightfield takes 2 minutes for an entire 96-well plate. A tumorsphere viability application is done in 7 minutes for 96-well plate.

### The Celigo will help:

- Save time by eliminating the need to use microscopy on a well-by-well basis
- Increase quality control standards by providing new quantifiable metrics for plateability, and cell health using data from whole well images
- Scale and validate assays for hepatobiliary cells or primary hepatocytes derived from many different sources and species
- Expand your scope of work to physiological relevant models for 3D & Co-culture
- Gain a competitive advantage to stay ahead of the drug discovery and development pack

### Plate Scan Times

Plate type	Scan area	Applications	Resolution (µm/pixel)	Typical time (min)
1536-well	Whole well	Confluence	2	6 min
384-well	1 FOV	Confluence	2	2 min
384-well	Whole well	Confluence	2	5 min
96-well	Whole well	Confluence	2	3.5 min
96-well	Whole well	Target 1	1	5 min
24-well	Whole well	Target 1 + Mask	1	16 min
24-well	Whole well	Confluence	2	10 min
6-well	Whole well	Confluence	2	11 min

“Celigo is relatively simple to use. It’s self-explanatory, reliable and the images you get out of it are nice, underlining your results. You can make nice presentations with the Celigo images.

## Celigo specifications

<b>Software</b>	Proprietary image acquisition and processing software Powerful analysis software/computer workstation Microsoft Windows				
<b>Illumination/optics</b>	1 LED-based enhanced brightfield imaging channel with uniform well illumination 4 LED-based fluorescent channels Proprietary F-theta lens with superior well edge-to-edge contrast Galvanometric mirrors for fast imaging of large areas Large chip CCD camera (2024x2024 pixels) 1, 2, 4 or 8 $\mu\text{m}$ /pixel resolution				
<b>Fluorescent channels</b>	Channel	Excitation	Dichroic	Emission	Typical dyes
	Blue	377/50	409	470/22	Hoechst, DAPI
	Green	483/32	509	536/40	FITC, Calcein, GFP, AlexaFluor® 488
	Red	531/40	593	629/53	R-PE, PI, Texas Red, AlexaFluor® 568
	Far-Red	628/40	660	688/31	DRAQ5®, AlexaFluor® 647
<b>Plate compatibility</b>	6, 12, 24, 48, 96, 384, 1536 well plates (black, white and clear wall plates) T-25 and T-75 flasks Slides and cell array plate profiles available upon request				
<b>High-speed imaging</b>	Less than 2 minutes per 384-well plate				
<b>Weight and dimensions</b>	Dimensions: 19.5 "W x 16 "H x 24 "D (49.5 cm x 40 cm x 61 cm) Weight: 117 lbs. (53 kg)				
<b>Power requirements</b>	110-220 VAC 50-60 Hz				
<b>Regulatory compliance</b>	CE marking				
<b>Focusing modes</b>	Hardware-based auto focus, image-based auto focus, manual focus				



Instrument	Catalog number	
Celigo 5 channel ( BR, Green, Red, Blue, Far Red)	200-BFFL-5C	
Plates or counting chambers		
Revvity <sup>3D</sup> 96-well Ultra-low attachment treated round bottom multi-well plates	ULA-96U-010; ULA-96U-020; ULA 96U-O520	
Revvity <sup>3D</sup> 384-well Ultra-low attachment treated round bottom multi-well plates	ULA-384U-010; ULA-384U-020; ULA 384U-O520	
Revvity Cellometer/Hepatometer Counting chambers	CHT4-SD100-002	
Reagent Kits		
ViaStain™ AOPI Staining Solution	CS2-0106-5mL	CS2-0106-25mL
ViaStain™ Calcein AM/Hoechst/PI Viability kit	CSK-V0006-1	
ViaStain™ Live Caspase 3/7 Detection for 2D/3D Culture with Hoechst	CSK-V0003-1	

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