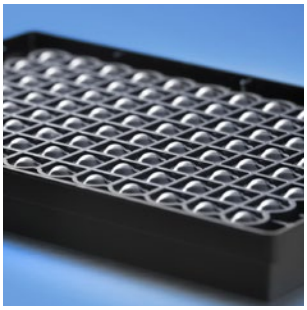
Corning® Spheroid Microplates

User Guide

CORNING



In vitro 3D cell culture models are widely recognized as more physiologically relevant systems compared to 2D formats. The 3D models reflect more accurately the complex *in vivo* microenvironment and have been used in many research areas, such as cancer biology[1], hepatotoxicity[2], neurology[3], pancreatic studies[4], nephrology[5], and stem cell biology[6]. These studies have revolutionized our understanding of cellular behavior both in culture and *in vivo*. However, adoption of 3D cell culture models in high throughput screening (HTS) platforms has been slow due to the limitations of the current technologies. Problems include increased variability, low throughput, difficulty to automate, and high cost[1].

Corning® spheroid microplates are multiple well, cell culture plates with opaque walls and clear, round well-bottom geometry. The proprietary Corning Ultra-Low Attachment surface coating, which is hydrophilic, biologically inert and non-degradable, is covalently attached to the interior surface of the well-bottom. The unique design of the well-bottom enables highly reproducible growth of 3D cell spheroid cultures. The opaque side walls and proprietary gridded plate bottom design reduce well-to-well cross-talk and background fluorescence/luminescence. Spheroids may be generated, cultured, and assayed for fluorescent or luminescent signals in the same plate without the need for transferring the spheroids. Currently, Corning spheroid microplates are available in both 96-well (Corning Cat. Nos. 4520 and 4515) and 384-well (Corning Cat. Nos. 3830 and 4516) formats. Both formats are automation friendly.

The protocol below describes a basic method for generating and culturing multicellular spheroids. Plating volumes and densities are cell line and downstream application dependent, therefore assay specific optimization of conditions is recommended.

Setting Up Cell Cultures

Initial plating densities for spheroid formation depend on factors such as cell-type, duration of growth phase in a spheroidal format and the desired size of spheroid at the time of assessment.

Depending on the cell lines to be cultured and downstream processes, starting volumes can range from 75 to 200 µL per well (maximum working volume: 300 µL) for the 96-well format and 25 to 75 µL per well (maximum working volume: 90 µL) for the 384-well format.

1. Prepare single cell suspension at the desired seeding concentration; cells can come from fresh cultures or from frozen vials.

   Note: If necessary to achieve a single cell suspension, cells can be passed through a 40 µm cell strainer (Corning Cat. No. 431750) or a 5 mL round bottom, polystyrene tube, with cell strainer snap cap (Corning Cat. No. 352235).

2. Dispense cell suspension into wells. This step can be achieved by using either manual multichannel pipettor or automated dispensing systems.

   Note: During manual seed, ensure pipet tips do not scratch the bottom or sides of the wells to avoid damaging the Ultra-Low Attachment surface coating.

3. Following dispensing, the plates can be covered then transferred to incubation step. (Set humidified incubator to 37°C and 5% CO_2 for most mammalian cultures).

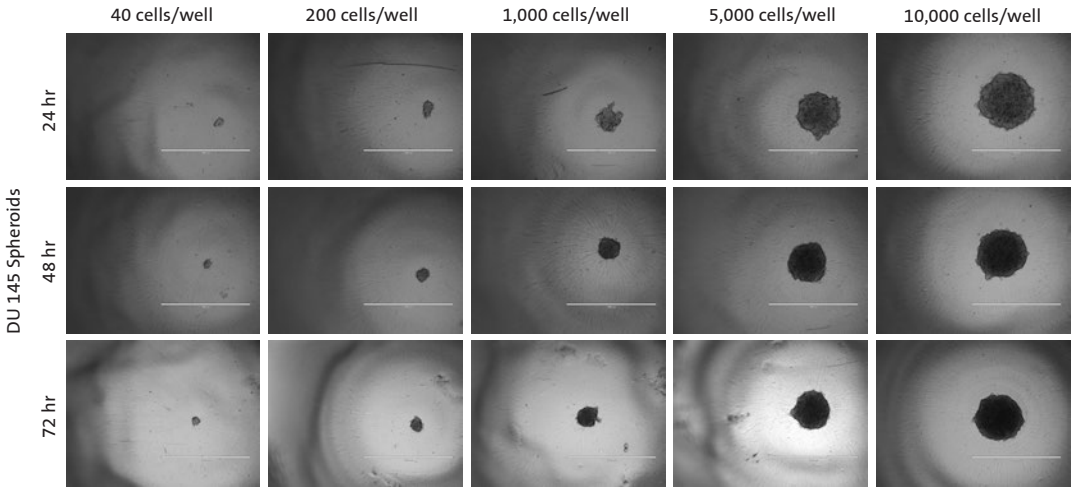
   Note: Most cell lines will form spheroids within a 24-hour period. Some cell lines may require a 15-minute incubation post-seed or a quick pulse spin in a centrifuge in order to help cells congregate faster. Optimization of the protocol is recommended.

4. Daily monitoring of spheroid formation and growth can be easily done using any microscope.
5. Depending on the cell line and the duration of growth phase in a spheroidal format, a re-feeding step may be necessary. Spheroids can be fed by adding fresh media to wells or by removing spent media and dispensing fresh media into the wells.
   a. Aspiration and addition of media can be done manually or using an automated liquid handling system.
   b. In order to leave spheroids undisturbed during media changes, sides of wells should be used to remove and add media.
   c. It is recommended that 10 to 20 µL volume be left in the well during media change.
6. Assay/process spheroids in microplate following recommended assay steps, no transfer step required.

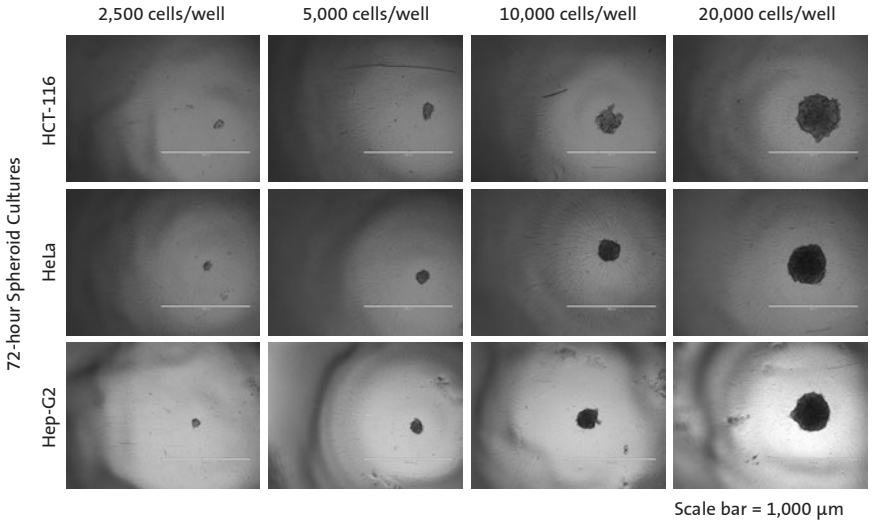
Examples of Multicellular Spheroids in Corning® Spheroid Microplates

Uniform, single mulicellular spheroids (MCS) generated per well.

DU 145 Spheroids in a 96-well Corning Spheroid Microplate

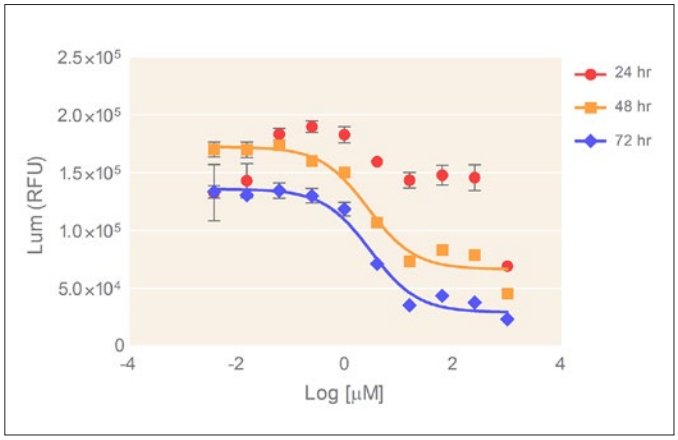


Hep-G2, HeLa, and HCT-116 Spheroids in a 384-well Corning Spheroid Microplate



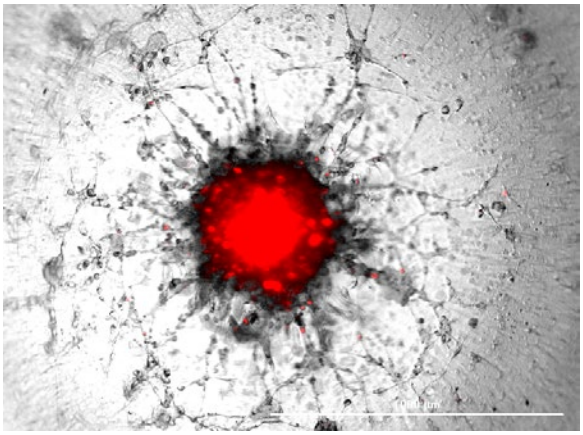
Examples of Assay Using Corning® Spheroid Microplates

Cell proliferation and cytotoxicity assay screening using CellTiter-Glo® 3D Cell Viability Assay (Promega Cat. No. G9683).



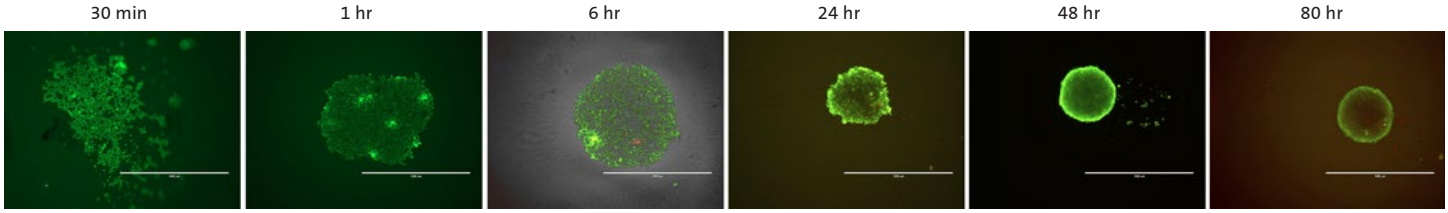
DU 145 spheroid dose response to Doxorubicin over a 72-hour treatment in a 384-well Corning spheroid microplate.

Tumor invasion assay using Cytation™ Cell Imaging Multi-Mode Reader and Gen5™ Data Analysis Software (BioTek Instruments, Inc.)



MDA-MB-231/Fibroblast tumor invasion in a 96-well Corning spheroid microplate imaged using the Cytation Cell Imaging Multi-Mode microplate reader. Image reprinted, courtesy of BioTek Instruments, Inc.

Fluorescence microscopy using LIVE/DEAD® Viability/Cytotoxicity Kit (Life Technologies, Cat. No. L3224).



Within the hour, cells form a loose aggregate.

Cells pool into a more defined aggregate.

Tight spheroid with defined edges.

Center of spheroid shows initial signs of necrosis.

HT-29 spheroid formation over a 3-day period in a 96-well Corning spheroid microplate.

Plate Dimensions to Support Instrumentation Setup

	Cat. No.	Well Volume (µL)	Well Depth (mm)	Well Diameter (top/bottom) (mm)	Plate Length (mm)	Plate Width (mm)	Plate Height (mm)	A1 Row Offset (mm)	A1 Column Offset (mm)	Well Center to Well Center Spacing (mm)	Flange or Skirt Height (mm)	Stack Height	Well Bottom Elevation (mm)	Well Bottom Thickness (mm)	Distance to Bottom of Plate
96-well	4520 and 4515	300	12.36	6.85/6.35	127.6	85.5	14.2	11.2	14.27	9	6.096	13.12	1.86	0.0875	1.86
384-well	3830 and 4516	90	12.54	3.63/2.82	127.6	85.5	14.2	8.99	12.13	4.5	2.41	12.95	1.81	0.0875	1.81

Corning® Spheroid Microplate FAQ

Q: How many spheroids are generated per well?

A: The Corning spheroid microplate was designed to generate single, uniform sized spheroids in every well.

Q: How do I control the size of the spheroids?

A: By altering the initial seeding density, the diameter of the spheroids in the culture can be controlled.

Q: Are there any special storage conditions?

A: No. The Corning Ultra-Low Attachment surface is a stable, noncytotoxic, nondegradable, biologically inert hydrogel coating that requires no special storage or handling conditions.

Q: What is the advantage of using a round bottom vs. a flat bottom microplate for forming spheroids?

A: The unique round well bottom design of the Corning spheroid microplate generates single, uniform spheroids, that are centered in each well. This technology is a reliable tool for 3D screening. Flat bottom wells generate multiple, non-uniform sized spheroids that are not evenly distributed and lack reproducibility across wells. Because of the variation from well to well, the technology does not lend itself to high throughput screening.

Q: What are the dimensions of the plate for automation programing?

A: Corning spheroid microplates adhere to the standard ANSI/SBS footprint dimensions for 96-well and 384-well microplate formats. A plate dimensions guide is provided with this user manual.

Q: What are the plate dimensions with respect to imaging, e.g., the bottom thickness?

A: Well bottom thickness for both 96-well and 384-well formats is 0.0875 mm. Please refer to the plate dimension guide for more information.

Q: How do I change media when feeding?

A: For a media change you need to remove a portion of the spent media from the wells and replace it with fresh media. To leave the spheroids undisturbed, we recommend performing this media change off-center using the sides of wells to remove and add media.

A 50/50 media exchange will be the easiest. For example, to remove 50% of media from a 96-well format with a 200 µL starting volume, aspirate 100 µL of spent media from the wells, and replace with 100 µL of fresh media.

For a full media change or wash step for either the 96-well or 384-well format, we recommend leaving behind a minimum of 10 µL per well. Use the plate dimension guide to program liquid handling instruments to be 2mm to 3 mm above the bottom of the wells and to be slightly off-center.

Q: Will evaporation be an issue, like with the hanging drop technique?

A: No. Evaporation is less of an issue because the spheroids are generated inside the wells. For long-term cultures, especially in the 384-well format, we recommend the use of breathable sealing tape (Corning Cat. No. 3345) during incubation periods.

Q: What imagers have been validated for compatible use with Corning spheroid microplates?

Manufacturer	Instrument Name
BioTek® Instruments, Inc.	Cytation® cell imaging multi-mode reader
Essen Bioscience	IncuCyte ZOOM®
Molecular Devices	ImageXpress® Micro XLS automated imaging system
Nexcelom	Celigo® S image cytometer
TTP Labtech	Acumen® Cellista laser scanning image cytometer

Q: Are there any issues when reading plates while using fluorescent/colorimetric agents?

A: No, Corning® spheroid microplates are specifically designed for assay use. The plates feature optically clear, round bottom wells with a black opaque microplate body. The plate design also includes a unique well shield to minimize well-to-well cross-talk.

We recommend using Promega CellTiter-Glo® 3D Cell Viability Assay for cell proliferation and cytotoxicity assay screening.

Q: Where can I find application notes for Corning spheroid microplates?

A: Corning technical literature can be found online at www.corning.com/lifesciences/cellbasedassays

References

1. Vinci, et al. 2012. Advances in Establishment and Analysis of Three Dimensional Tumor Spheroid-based Functional Assays for Target Validation and Drug Evaluation. BMC Biology 10:29.
2. Godoy P., et al. 2013. Recent Advances in 2D and 3D *In Vitro* Systems Using Primary Hepatocytes, Alternative Hepatocyte Sources and Non-parenchymal Liver Cells and Their Use in Investigating Mechanisms of Hepatotoxicity, Cell Signaling and ADME. Arch. Toxicol. 87(8):1315-1530. Pubmed/23974980.
3. Lai, et al. 2011. Neural Cell 3D Microtissue Formation is Marked by Cytokines' Up-regulation. PLoS One 6(10):e26821.
4. Jun Y. 2013. 3D Co-culturing Model of Primary Pancreatic islets and Hepatocytes in Hybrid Spheroid to Overcome Pancreatic Cell Shortage. Biomaterials 34(15):3784-3794.
5. Astashkina, et al. 2012. A 3-D Organoid Kidney Culture Model Engineered for High-Throughput Nephrotoxicity Assays. Biomaterials 33:4700-4711.
6. Sasai Y. 2013. Next-Generation Regenerative Medicine: Organogenesis from Stem Cells in 3D Culture. Cell Stem Cell 12(5):520-530.

For more specific information on claims, visit the Certificates page at **www.corning.com/lifesciences**.

For additional product or technical information, please visit **www.corning.com/lifesciences** or call 1.800.492.1110. Outside the United States, call 978.442.2200.

Corning Incorporated
Life Sciences
836 North St.
Building 300, Suite 3401
Tewksbury, MA 01876
t 800.492.1110
t 978.442.2200
f 978.442.2476
www.corning.com/lifesciences

Worldwide Support Offices

ASIA/PACIFIC

Australia/New Zealand
t 61 427286832

China
t 86 21 3338 4338
f 86 21 3338 4300

India
t 91 124 4604000
f 91 124 4604099

Japan
t 81 3-3586 1996
f 81 3-3586 1291

Korea
t 82 2-796-9500
f 82 2-796-9300

Singapore
t 65 6572-9740
f 65 6861-2913

Taiwan
t 886 2-2716-0338
f 886 2-2516-7500

EUROPE

France
t 0800 916 882
f 0800 918 636

Germany
t 0800 101 1153
f 0800 101 2427

The Netherlands
t 31 20 655 79 28
f 31 20 659 76 73

United Kingdom
t 0800 376 8660
f 0800 279 1117

All Other European Countries
t 31 (0) 20 659 60 51
f 31 (0) 20 659 76 73

LATIN AMERICA
e grupoLA@corning.com

Brasil
t (55-11) 3089-7400
f (55-11) 3167-0700

Mexico
t (52-81) 8158-8400
f (52-81) 8313-8589

CORNING | FALCON AXYGEN GOSSELIN PYREX