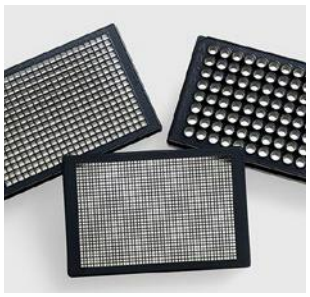
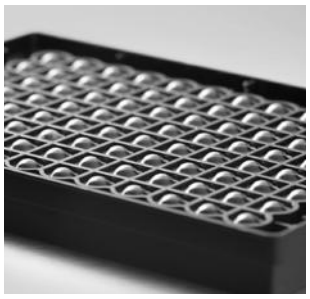
Corning® Spheroid Microplates

User Guide







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 96-well (Corning Cat. Nos. 4520 and 4515), 384-well (Corning Cat. Nos. 3830, 3830BC, and 4516), and 1536-well (Corning Cat. No. 4527) formats. All 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:

96-well:	75 to 200 µL per well (maximum working volume: 300 µL)
384-well:	25 to 75 µL per well (maximum working volume: 90 µL)
1536-well:	5 to 10 µL per well (maximum working volume: 14 µL)

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.

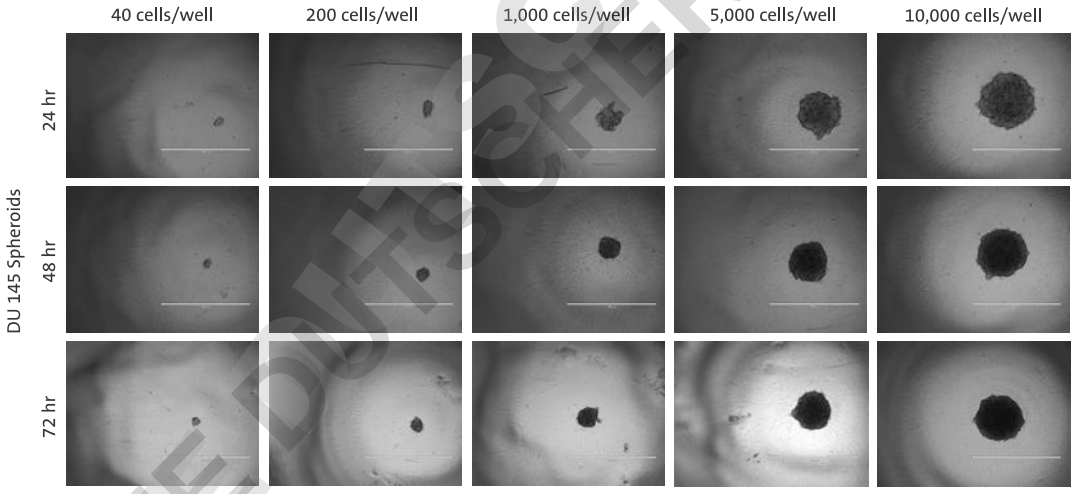
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4. Daily monitoring of spheroid formation and growth can be easily done using any microscope.
5. Depending on the cell line and the duration of growth phase in a spheroidal format, a re-feeding step may be necessary. Spheroids can be fed by adding 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 for the 96-well and 384-well formats and 5 µL for the 1536-well format.
6. Assay/process spheroids in microplate following recommended assay steps, no transfer step required.

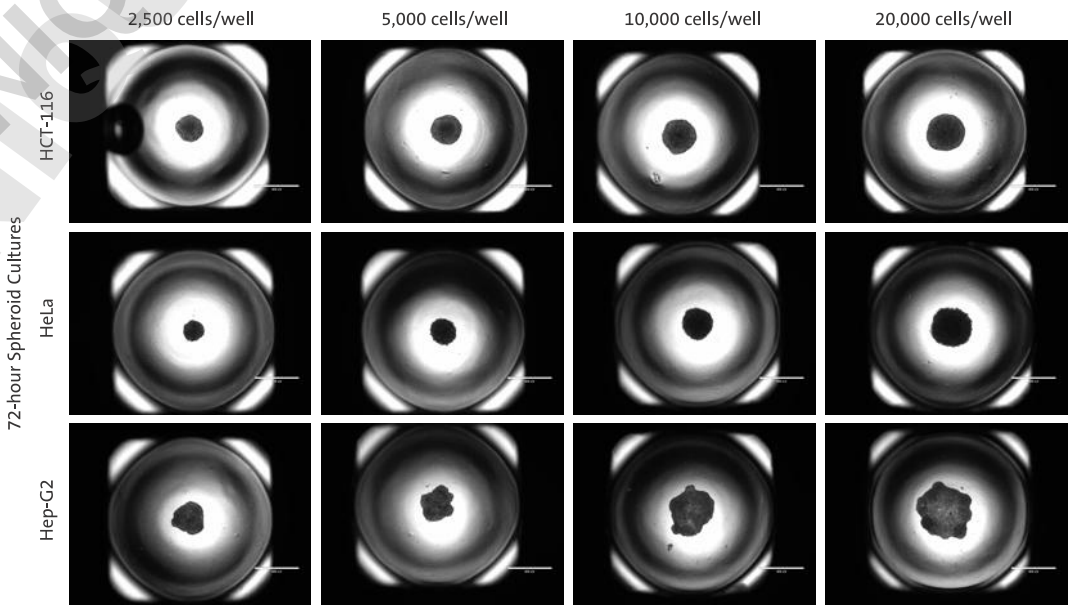
Examples of Multicellular Spheroids in Corning® Spheroid Microplates

Uniform, single multicellular 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

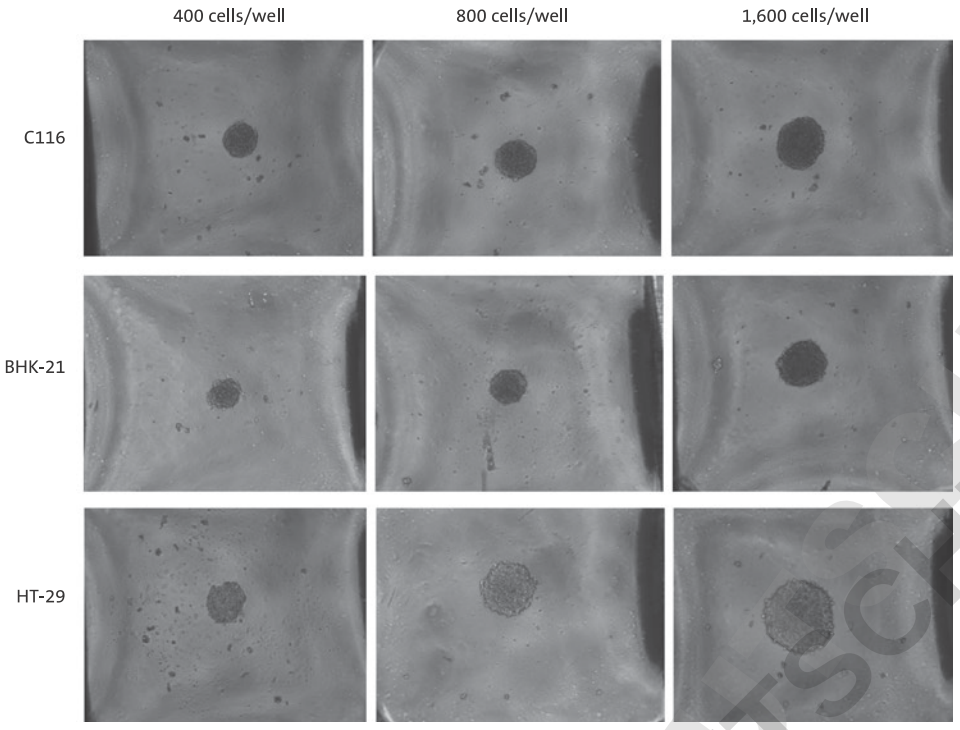


Scale bar = 1,000 µm

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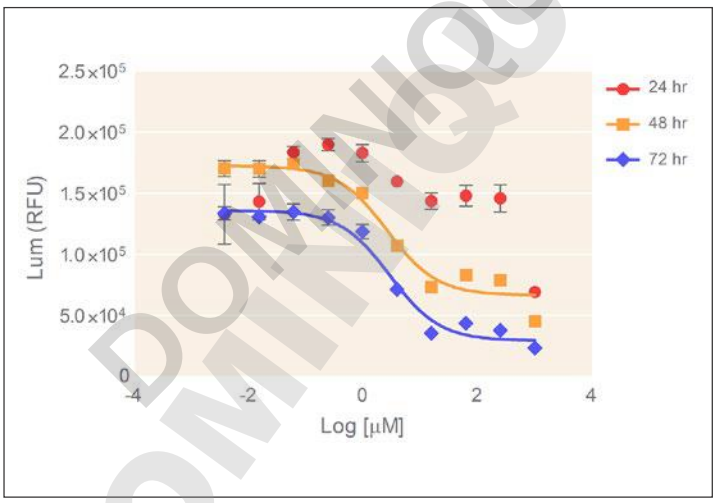
Examples of Multicellular Spheroids in Corning® Spheroid Microplates (Continued)

48-hour Spheroid Cultures – Microscope Images Captured at 4X Magnification, in a 1536-well Corning Spheroid Microplate



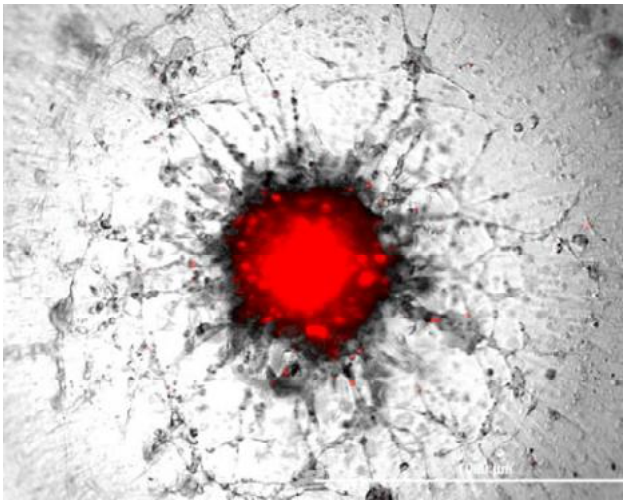
Examples of Assay Using Corning Spheroid Microplates

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)

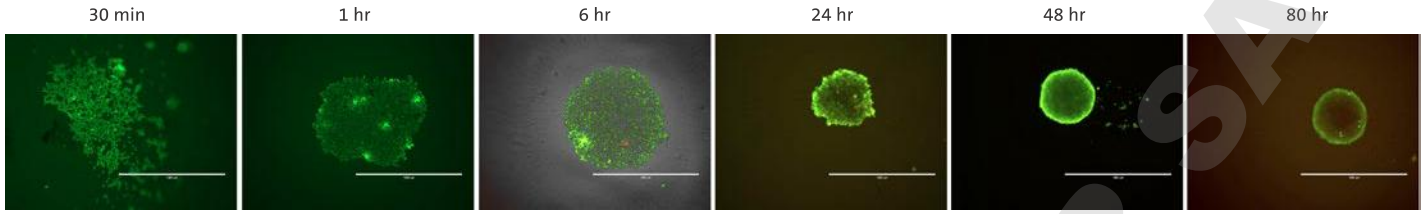


MDA-MB-231/Fibroblast tumor invasion in a 96-well Corning spheroid microplate imaged using the Cytation Cell Imaging Multi-Mode microplate reader. Image reprinted, courtesy of BioTek Instruments, Inc.

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Examples of Assay Using Corning® Spheroid Microplates (Continued)

Fluorescence microscopy using LIVE/DEAD® Viability/Cytotoxicity Kit (Thermo Fisher Cat. No. L3224).



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

72-hour HeLa/GFP Spheroids

Plate scan at 4X using CellInsight™ CX7 High-Content Screening Imager, in a 1536-well Corning Spheroid Microplate



Microplate Dimensions to Support Instrumentation Setup

	Spheroid Microplate (Corning 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)	Well Bottom Area (cm^2)	Distance to Bottom of Plate
96-well	4515, 4520	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	N/A	1.86
384-well	4516, 3830, 3830BC	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	N/A	1.81
1536-well	4527	14.67	6.2	1.7/1.45 (Sq)	127.6	85.5	8.0	7.87	11.01	2.25	2.16	6.70	1.81	0.1016	N/A	1.81

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Corning® Spheroid Microplate FAQ

1. **How many spheroids are generated per well?**

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

2. **How do I control the size of the spheroids?**

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

3. **Are there any special storage conditions?**

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

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

   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.

5. **What are the dimensions of the plate for automation programing?**

   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.

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

   Well bottom thickness for both 96-well and 384-well formats is 0.0875 mm and 0.1016 mm for the 1536-well format. Please refer to the plate dimension guide for more information.

7. **How do I change media when feeding?**

   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, remove 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. Automation will be required for liquid handling of the 1536-well format.

8. **Will evaporation be an issue, like with the hanging drop technique?**

   No. Evaporation is less of an issue because the spheroids are generated inside the wells. For long-term cultures, especially in the 384-well and 1536-well formats, we recommend the use of breathable sealing tape (Corning Cat. No. 3345) during incubation periods.

9. **What imagers have been used with Corning 96- and 384-well spheroid microplates?**

Manufacturer	Instrument Name
BioTek® Instruments	Cytation® cell imaging multi-mode reader
Molecular Devices	ImageXpress® Micro XLS automated imaging system
Nexcelom	Celigo® S image cytometer
Thermo Fisher	CellInsight™ CX7 high content system
TTP Labtech	Acumen® Cellista laser scanning image cytometer

10. **Are there any issues when reading plates while using fluorescent/colorimetric agents?**

    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.

11. **Where can I find application notes for Corning spheroid microplates?**

    Corning technical literature can be found online at **www.corning.com/lifesciences**.

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