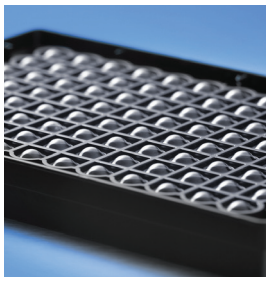
Corning® 96-well Spheroid Microplates for 3D Cell Culture

Protocol

CORNING



With a novel proprietary design, Corning 96-well spheroid microplates are ideal for generating and analyzing 3D multicellular spheroids in the same microplate. The microplates offer the following benefits:

- The Corning Ultra-Low Attachment (ULA) surface is a covalently bound hydrogel layer that reduces cell attachment and aides in the promotion of multicellular spheroids.
- Novel well geometry aids in the generation of single, uniform, and reproducible 3D multicellular spheroids across all wells, which enables automated visualization.
- The black opaque microplate body shields the optically clear, round-bottom well from well-to-well cross talk.
- Spheroids can be cultured and assayed in the same microplate without the need for transfer to a new microplate.

Cat. No.	Description	Qty/Pk	Qty/Cs
4520	96-well spheroid microplate, black/clear round-bottom, ULA surface, sterile	10	50
4515	96-well spheroid microplate, black/clear round-bottom, ULA surface, sterile	5	5

Note: The protocol below describes a basic method for generating and culturing multicellular spheroids with HT-1080 cells. This is a generalized protocol and could be adapted to many different cell types. Plating volumes and densities are cell type-dependent and downstream application-dependent; therefore optimization is highly recommended prior to use for any assay.

Reagents and Materials

- HT-1080 cell line (ATCC CCL-121, human fibrosarcoma cell line)
- T-25 flask (Corning Cat. No. 430639)
- Minimum Essential Medium (MEM, Corning Cat. No. 10-009-CV)
- Fetal Bovine Serum (FBS, Corning Cat. No. 35-076-CV)
- 0.05% Trypsin/EDTA (Corning Cat. No. 25-051-CI)
- Phosphate Buffered Saline (PBS, Corning Cat. No. 21-040-CVR)
- 96-well spheroid microplate, black/clear round-bottom (Corning Cat. No. 4520)
- Trypan blue stain 0.4% (Invitrogen Cat. No. T10282)
- Countess™ cell counting chamber slides (Invitrogen Cat. No. C10283)
- Countess automated cell counter (Invitrogen Cat. No. C10281)
- DMSO (Corning Cat. No. 25-950-CQC)
- Calcein AM fluorescent dye (Corning Cat. No. 354216)

Procedure

Cell Preparation

1. Recover the HT-1080 cells (human fibrosarcoma cell line) and culture with MEM medium supplemented with 10% FBS.
2. Passage the cells two or more times as required to reach a stable status. Ensure that cells have a typical healthy morphology by examining the cell cultures with an inverted microscope.
3. After cells are stable and healthy, grow the cells to adequate density (80% to 90% confluence) in a T-25 flask for seeding.

Cell Seeding/Cell Harvest

1. Remove the culture medium. Rinse the cells once with 5 mL PBS.
2. Add 1 mL of 0.05% trypsin/EDTA to the cells. Ensure the trypsin coats the entire cell culture surface.
3. Incubate at room temperature for 3 to 5 min. (this is cell type-dependent) to detach the cells from the surface.
4. Terminate the trypsinization by adding an equal volume of MEM medium with 10% FBS.
5. Gently resuspend the cells and transfer the cell suspension into a 15 mL centrifuge tube.
6. Centrifuge at 100 x g for 5 min.
7. Discard the supernatant. Resuspend the cells in 2 mL of MEM medium with 10% FBS. Disperse the cells by pipetting up and down several times.

Cell Counting

1. Transfer 50 µL of the cell suspension into a 1.5 mL tube, add equal volume of 0.4% trypan blue, and mix gently.
2. Add 10 µL of the cell suspension-trypan blue mixture to the Countess™ cell counting chamber slide.
3. Read the cell density and viability with the Countess automated cell counter.

Cell Seeding into a Spheroid Microplate

1. Adjust the cell density to 1 x 10^5/mL. Seed 100 µL of this cell suspension into each well of the Corning 96-well spheroid microplate. The final cell density is 10K per well.

   Note: 10K cells per well is a recommendation. The initial plating densities and volume for spheroid formation may need optimization depending on cell type, duration of growth phase, and downstream processes.

2. Culture the cells in a humidified 37°C, 5% CO_2 incubator for the desired culture period.

   Note: HT-1080 cells will form spheroids within a 24-hour period. However, optimization for the culture period is highly recommended for different cell types, culture conditions, and downstream processes.

   Note: Depending on the cell line and the duration of the growth phase in spheroids, a re-feeding step may be necessary. It is recommended to leave 10 to 20 µL of the original solution in the well during the media change to avoid disturbing the spheroids.

Live Cell Staining and 3D Culture Observation

1. Spin down the Calcein AM powder to the tube bottom. Prepare 1 mM stock by adding 50 µL DMSO to 50 µg Calcein AM powder. Store at 4°C before use.

   Note: Calcein AM becomes damp easily. Use it in a dry environment.

2. Dilute the 1 mM Calcein AM stock with PBS to a final concentration of 100 µM working solution.
3. Add 10 µL of 100 µM Calcein AM working solution directly into each well with the cell spheroid in 100 µL medium. The final concentration of Calcein AM is 10 µM.

   Note: To avoid disturbing the spheroids, do not remove the medium from the well.

4. Incubate the microplate at 37°C for 60 min.
5. Observe the cell spheroids under a fluorescence microscope with an excitation wavelength at ~490 nm.

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