User Guide

Millicell® Ultra-low Attachment Plates

96-well, U-bottom, clear polystyrene

MC96ULA20

Product Description

The Millicell® Ultra-low Attachment (ULA), 96-well, U-bottom, clear plates are used to generate homogeneous spheroid cultures.

Each well contains a specialized ULA ultra-hydrophilic polymer coating that enables spontaneous spheroid formation of uniform size and shape, and prevents cells from adhering. Coupled with the U-shaped round bottom, the Millicell® ULA Plates enable the production, culture, and analysis of spheroids and other 3D cell structures. The initial cell aggregate size is highly uniform and can be controlled by adjusting the initial cell seeding. The clear Millicell® ULA Plates offer great visibility of aggregate formation and growth of the 3D cultures over time.

Millicell® ULA Plates promote scaffold free, self-assembly of spheroid formation. The Millicell® ULA Plates have high optical clarity making them highly suitable for brightfield imaging and confocal microscopy.

Millicell® ULA Plates are gamma irradiated in packaging.

Features

- Millicell® ULA Plates have well numbers indicated on both top and bottom of each column.
- The frosted effect on the plate enhances the ability to read the column and row indication labels.
- The plate has an opening on the on the long side of the bottom of the plate for easier grip.
- The Millicell® ULA Plate lid fits the plate with rings designed to frame the top of each well to impede evaporation.
- The round U-shaped bottom of the Millicell® ULA Plate provides optimum spheroid growth.
  - Stable, non-cytotoxic and cell non-adhesion surface
  - Easy to handle, compatible with liquid robotic system
  - Round bottom wells have high optical clarity making them suitable for bright field imaging and confocal microscopy.
  - Uniform single spheroid formation in each well

3D Spheroid Applications

- Fluorescent Image Analysis, cell division in spheroids
- Fluorescent Image Analysis, live dead cell assay of spheroids
- Evaluations of Drug Efficacy, the response of co-cultured spheroids to drugs
- Analysis of Anticancer Drugs, targeting the interior of spheroid
- Evaluation of Drug Efficacy, quantitative evaluation of spheroid size
- Evaluation of Drug Efficacy, the response of spheroids to a drug
- Cell Counting, measuring the number of cells in spheroids

Storage

Store Millicell® ULA Plates at room temperature and low humid location away from direct sunlight. Do not use if package is wet or damaged.

Chemical Resistance

Organic Solvent

Time	1 hour			5 hours		
Percent Solvent	10%	50%	100%	10%	50%	100%
Methanol	✓	✓	✓	✓	✓	✓
Ethanol	✓	✓	✓	✓	✓	✓
2-propanol	✓	✓	✓	✓	✓	✓
Glycerol	✓	–	–	✓	–	–
Acetonitrile	✓	✓	✓	✓	✓	✓
Acetone	✓	✓	✓	–	–	–
DMSO	✓	–	–	–	–	–
Mercaptoethanol	✓	–	–	✓	–	–

Surfactant	0.1%
CHAPS	✓
Triton X	✓
Tween20	✓
SDS	✓

Legend	
passed testing	✓
uncomfirmed	–

Specifications

Plate Type	96-well
Plate Material	Clear polystyrene
Lid Material	Clear polystyrene
Well Volume	Max 300 µL <250 µL recommended
Well Depth	10 mm
Well Diameter Top/Bottom	7.0/6.1 mm
Plate Length	127.5 mm
Plate Width	85.9 mm
Plate Height	14 mm
A1 Row Offset	11.45 mm
A1 Column Offset	14.25 mm
Well Center to Well Center Spacing	9 mm
Flange or Skirt Height	3.5 mm
Stack Height	12.9 mm
Well Bottom Elevation	4 mm
Well Bottom Thickness	1.1 mm
Distance to Bottom of Plate	2.9 mm

Compatible with brightfield and fluorescence imaging systems.

Directions for Use

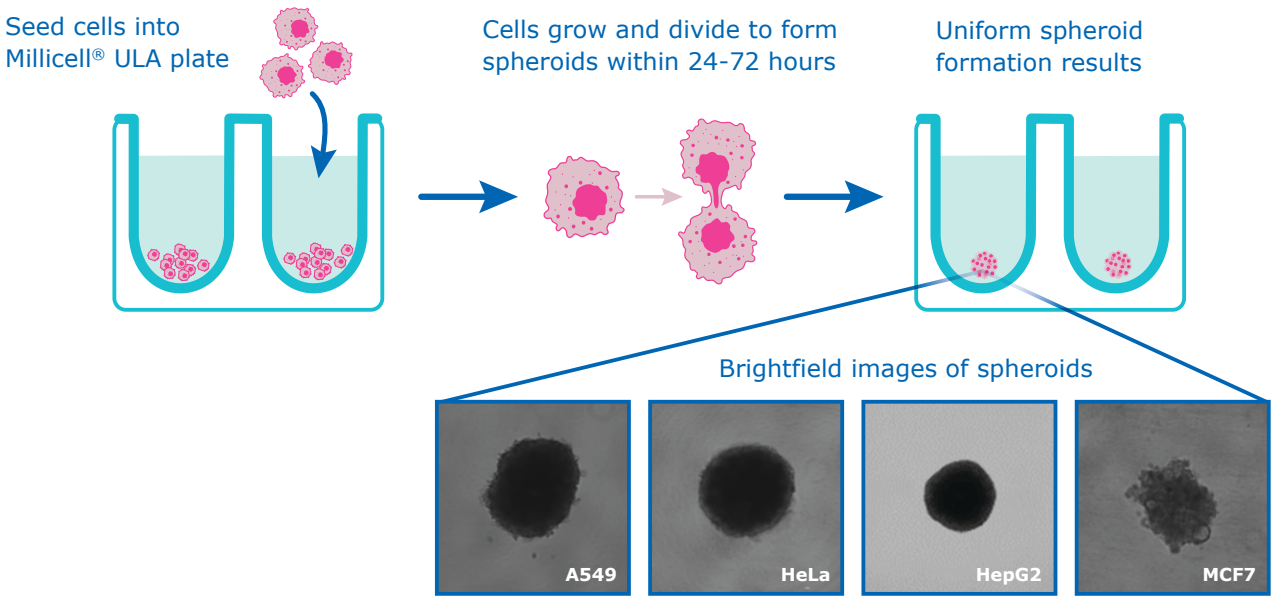
Please read the entire protocol before proceeding.

Preparation

Millicell® ULA Plates with ULA coating on the well surface, received gamma irradiated in its packaging. To prevent contamination, plates should be opened inside a bio-safety cabinet.

Generation of Cell Aggregates from a Single Cell Suspension

Multiple cell lines were shown to form single, centered spheroids in 96-well microplate formats. Brightfield images were taken after incubating samples for 72 hours (10x and 4x magnification).



Seeding Cells in wells

1. Culture cells in 2D cell culture flask until they are between 60-80% confluence.
2. Aspirate media from the culture flask.
3. Add sterile PBS for 3 minutes at room temperature.
4. Aspirate liquid from the culture flask.
5. Add Trypsin to the culture flask and incubate at 37 °C 5% carbon dioxide cell culture incubator.
6. Stop the trypsin reaction by adding equal parts complete cell culture media to the flask.
7. Collect liquid suspension and spin down in the centrifuge at 300 x *g* for 3 minutes.
8. Re-suspend cells in 1 mL of cell complete culture media.
9. Count cells using Trypan Blue Solution and a Millicell® Disposable Hemocytometer. Mix 90% trypan blue with 10% cell suspension (e.g., 180 µL trypan blue and 20 µL cell suspension).
10. Count live cells on the hemocytometer and calculate cells per milliliter.
11. Mix to create a single cell suspension and add desired cell suspension amount to 96-well microplate.

    Note: Millicell® ULA Plates can hold a maximum of 300 µL volume, but we advise <250 µL volume for best results.
12. Depending on the cell type and medium, cells will aggregate to form a single spheroid within 24-72 hours (about 3 days).

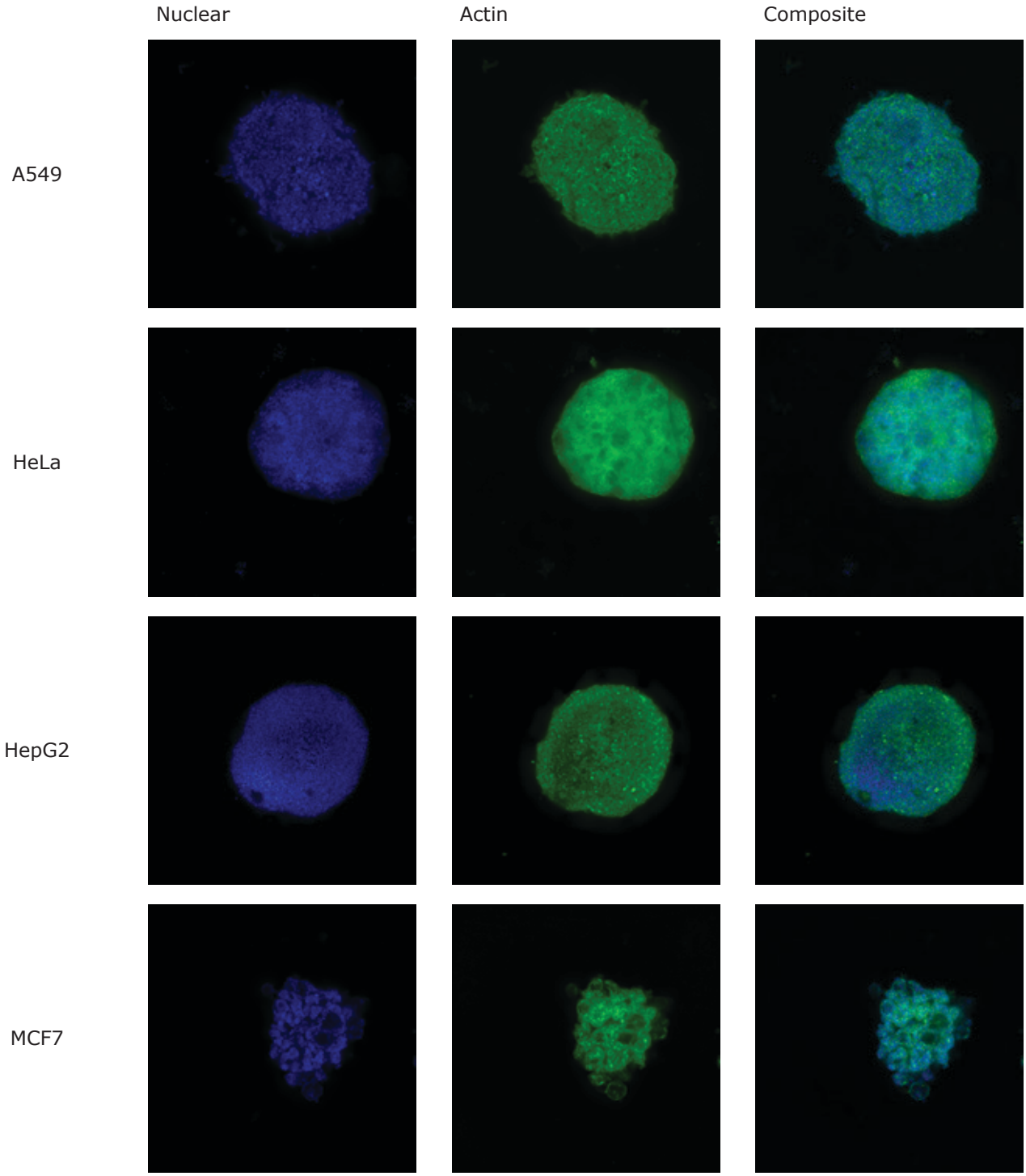
How to Change Medium

For best results perform half media changes to avoid disrupting spheroid formation (if total well volume is 200 µL, then aspirate 100 µL and replace with fresh 100 µL volume).

Imaging and Staining Spheroids

Spheroids may be generated, cultured, and assayed for fluorescent or luminescent signals in the same Millicell® ULA Plate without the need to transfer them to another plate. The unique U-shaped well geometry makes them suitable for automated imaging of spheroids for high content screening approaches.

Images below have been taken on the ImageXpress® Micro Confocal High-Content Imaging System with 10x objective.



In-well Assay Manipulation for Fixation and Staining Spheroid

1. (x3) Remove 100 µL from supernatant and add 100 µL wash buffer. Incubate at room temperature for 5 minutes.

   Note: For best results perform half media changes to avoid disrupting spheroid formation (if total well volume is 200 µL, then aspirate out 100 µL volume and replace with fresh 100 µL volume).

2. Remove 100 µL from supernatant. Add in 2x fixative (e.g., 8% PFA for a final concentration of 4% PFA) and incubate for 1 hour at 4 °C.

   Note: The fixative is made up as a 2x stock and added 1:1 with the residual volume in the well with the spheroid.

3. (x3) Remove 100 µL from supernatant and add 100 µL wash buffer. Incubate at room temperature for 5 minutes.

4. Remove 100 µL from supernatant. Add in primary antibodies and incubate for 1 hour in the dark at room temperature.

5. (x3) Remove 100 µL from supernatant and add 100 µL wash buffer. Incubate at room temperature for 5 minutes.

6. Remove 100 µL from supernatant. Add in secondary antibodies and incubate for 1 hour in the dark at room temperature.

7. (x3) Remove 100 µL from supernatant and add 100 µL wash buffer. Incubate at room temperature for 5 minutes.

8. Remove 100 µL from supernatant. Dilute stock DAPI in PBS to 1 µg/µL and add 100 µL to each well. Incubate overnight in the dark at 4 °C.

   Note: Wrap your plate with paraffin or other sealant to avoid evaporation of liquid.

9. (x3) Remove 100 µL from supernatant and add 100 µL wash buffer. Incubate at room temperature for 5 minutes.

10. Ready to image.

Troubleshooting

Problem	Cause(s)	Solution(s)
Single cell suspension aggregating/clumping	Sticky/clumpy cells	Use a 40 µm cell strainer to remove aggregates/clumps of cells
Spheroids not forming or multiple aggregates formed	Pipette touched the bottom or sides of the microwell when manually seeding	Reseed plate avoiding contact with the plate bottom or sides
	ULA coating damaged	Replace the plate
	Pre-existing aggregates in well	Start with a single cell suspension
	More time is required	Cells may take 24-72 hours depending on type. Optimization is required for each cell type
	Bacterial contamination	Check culture for bacteria and use antibiotics in media
Spheroids forming loose aggregates	Cell lines may vary	Fibroblast co-culture may be required
		Additional media supplementation such as methyl-cellulose may be required
		Optimization is normally required for each cell type
Losing spheroids during media changes	This is an ultra-low attachment plate, non-adhesion spheroids might be inadvertently removed with media aspiration.	Perform half media changes and micro-pipette gently
		Slowly aspirate liquid so not to disturb 3D cultures
		Do not set the pipette tip near the spheroid in the media
Spheroids are not forming uniform size 3D aggregates	Non-uniform starting single cell suspension	Ensure that you are mixing the starting single cell suspension before adding it to plate
	Heterogeneous co-culture cell mixture	Mix co-culture cell types well before dispensing into the plate
	Unknown starting quantity of cells	Count cells in a hemocytometer to add a known cell number to the plate
	Unequal media volume	Add equivalent volumes of media to each well

Product Ordering

To order, go to SigmaAldrich.com.

Description	Quantity	Catalogue Number
Millicell Ultra-low Attachment Plates, 96-well, U-bottom, Clear	20 pk	MC96ULA20
Trypan Blue Solution	50 mL	93595-50ML
Trypsin	100 mg	T2600000
Millicell® Disposable Hemocytometer	50 pk	MDH-2N1-50PK

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