User Guide

Millicell® Microwell Plates

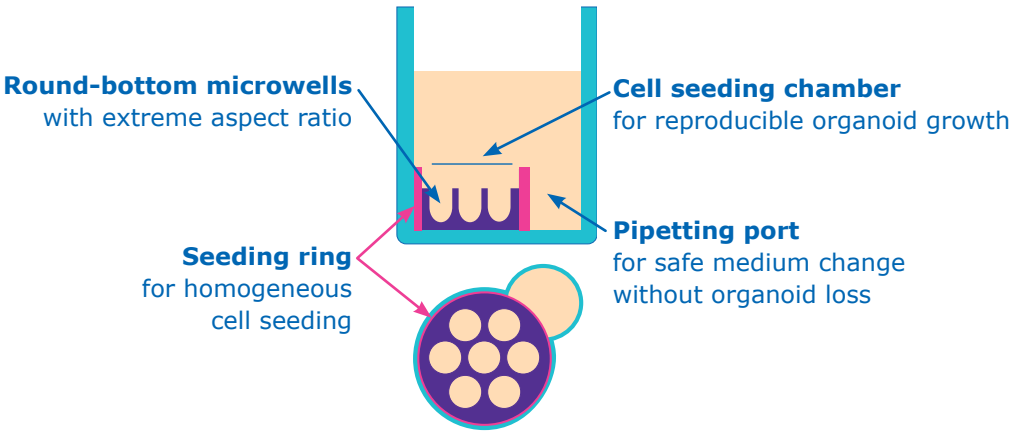
Hydrogel Microwell Arrays for Reproducible Long-term 3D Cell Cultures

MC96U4005
MC96U6005

Product Description

Millicell® Microwell Plates are used to generate homogeneous organoid cultures. Each well contains a standardized microstructured hydrogel array of U-shaped microwells that enable production, culture and analysis of organoids and other 3D cell structures. The initial cell aggregate size is highly uniform and can be controlled by adjusting the initial cell seeding density. Millicell® Microwell Plates are available in different microwell sizes to accommodate the initial aggregate size and the growth of the 3D cultures over time.

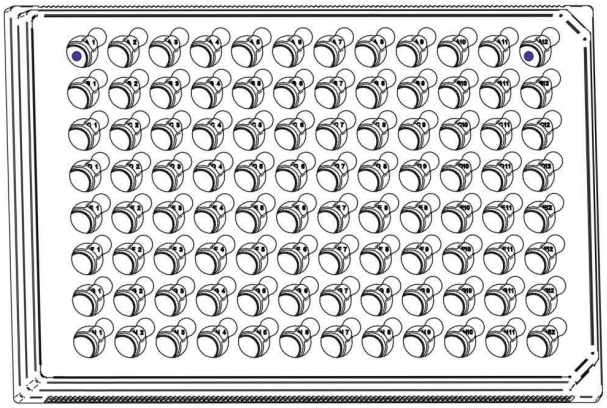
Millicell® Microwell Plate Features



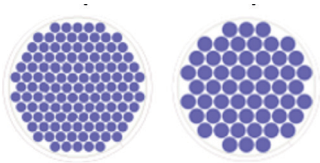
Millicell® Microwell Plate consists of an array of U-bottom shaped microwells in a polyethylene glycol (PEG) hydrogel. The microwells are surrounded by a seeding ring that separates the cell seeding chamber from the independent pipetting port.

Plate Format	96-well Plate	
Microwell Sizes (µm/well)	400	600
Number of Microwells	121*	55*
Working Volume (µL)	200	200
Plastic Bottom	Yes	Yes

* Shown are maximum number of microwells per well. Actual number of microwells may be up to 7% fewer.



400 µm **600 µm**

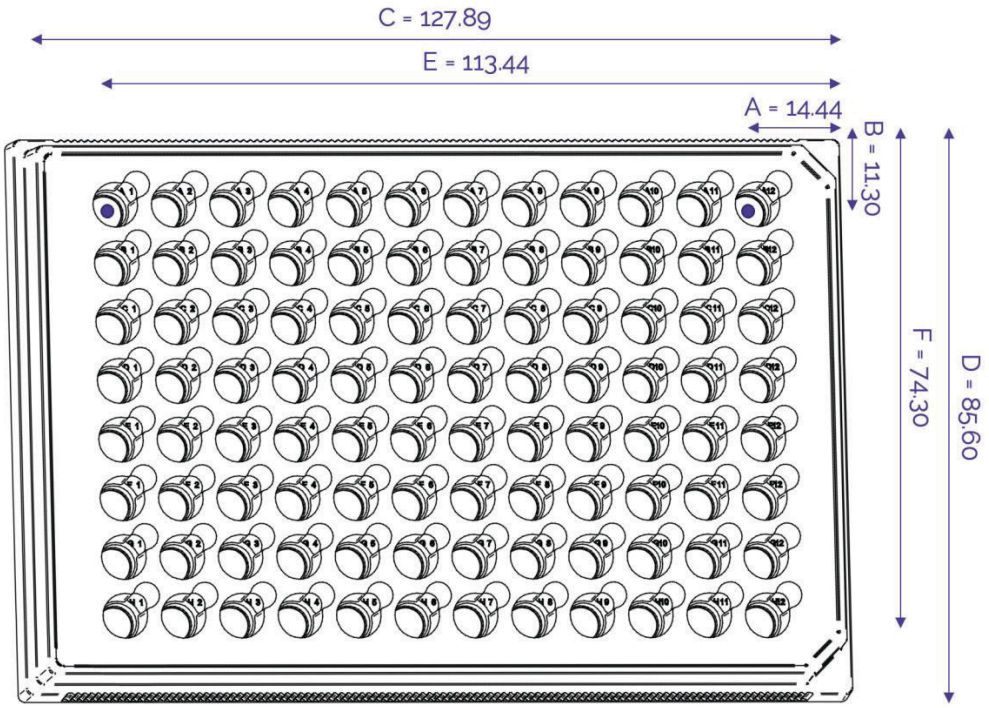


Storage and Stability

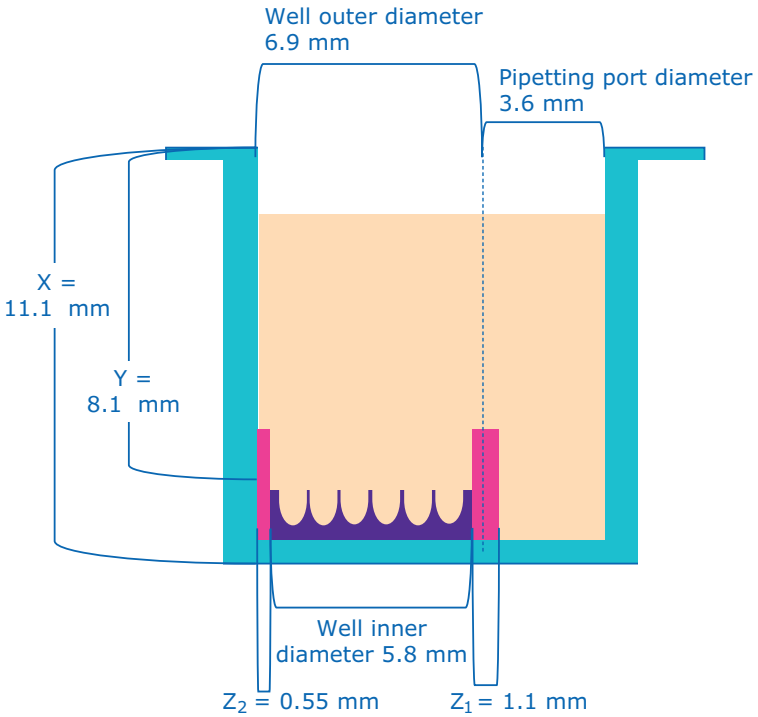
Store Millicell® Microwell Plates at 4-8 °C, away from direct sunlight.
The plates will be stable for 12 months after receipt.

Specifications

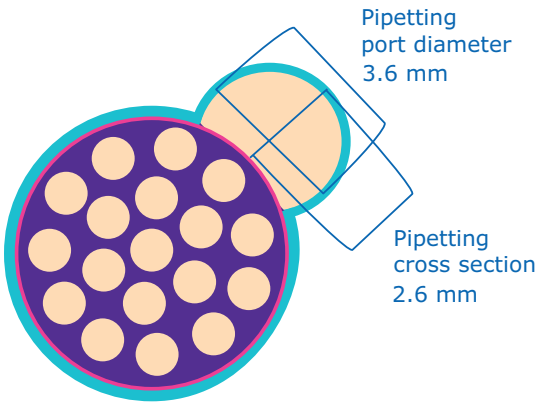
Measurements



Cross Section View



Top View



Millicell® Microwell Plate Dimensions to Support Instrument Setup

Millicell® Microwell Plate Dimensions	
Well Volume (µL)	200-300
Well Depth (mm)	11.10
Well Diameter top/bottom (mm)	6.86/5.40
Plate Length (mm)	127.90
Plate Width (mm)	85.60
Plate Height (mm)	14.45
A1 Row Offset (mm)	11.30
A1 Column Offset (mm)	14.44
Well Center to Well Center Spacing (mm)	9.00
Flange or Skirt Height (mm)	6.15
Stack Height (mm)	12.10
Well Bottom Elevation (mm)	2.35
Well Bottom Thickness (mm)	1.00
Microwell Bottom to Bottom of Plate (mm)	3.35 + (0.15 - 0.18)

Measurements for Use in ImageXpress® Microscopy

Metric	Measure
Number of Rows	8
Number of Columns	12
Well Shape	Circle
Well Bottom	Round
Plate Bottom	Continuous
Well Diameter (µm)	5400
Column Offset (µm)	14440
Row Offset (µm)	11300
Column Spacing (µm)	9000
Row Spacing (µm)	9000
Well Depth (µm)	11100
Plate Length (mm)	127.90
Plate Width (mm)	85.60
Plate Height (mm)	14.45

Directions For Use

Please read the entire protocol before proceeding.

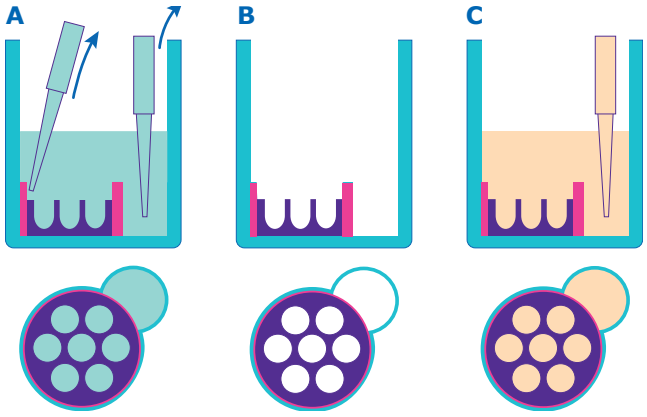
Preparation of Millicell® Microwell Plates

Millicell® Microwell Plates are shipped sterile and contain shipping buffer to keep the hydrogel arrays hydrated. The plates are vacuum sealed to avoid the buffer from leaking. Before use, sterilize the plate with its outer plastic wrapping with 70% alcohol. Remove the wrapping while keeping the plate leveled and place inside the TC hood. Then remove the lid and the sealing layer inside the lid.

1. Remove shipping buffer from both the pipetting port and the cell seeding chamber as illustrated (A-B).
2. Equilibrate the hydrogel microwell arrays in growth medium before seeding cells by adding 150 µL of growth medium into the pipetting port of each well of the plate (C). Alternatively, for precious medium, aspirate shipping buffer from the inner cell seeding ring and add 50 µL of medium to the cell seeding chamber.

   Tip: First attach a wide-bore pipette tip to an aspirator and remove the shipping buffer from the pipetting port. Then slide the pipette tip at an angle on the side of the well until you feel a resistance, which is the seeding ring, and aspirate from there without touching the hydrogel.

   Note: It is not necessary to completely remove the shipping buffer from the cell seeding chamber.
3. Incubate the Millicell® Microwell Plate for a minimum of 30 minutes at room temperature or in a 37 °C with 5% CO_2 incubator.



Millicell® Microwell Plate Preparation. Remove shipping buffer from the pipetting port (A-B). Add 150 µL of growth medium to the pipetting port (C).

Generation of Cell Aggregates from a Single Cell Suspension

4. Prepare a single cell suspension of your cells at the desired cell density in the desired growth medium. A minimum of 100 cells should be seeded into each microwell. The cell number should be adjusted according to the growth rate of your cells. The recommended seeding volume is 50 µL per well.

   Refer to the Cell Seeding Density table on page 5 to determine the cell seeding density required for a 100 cells per microwell. Alternatively, calculate using the formula as follows:

   Seeding density (cells/mL) = # of cells per microwell x # of microwells x 20

   Tip: For seeding a full Millicell® Microwell Plate, a minimum of 5 mL of single cell suspension +5% overage is required (total = 5.25 mL).

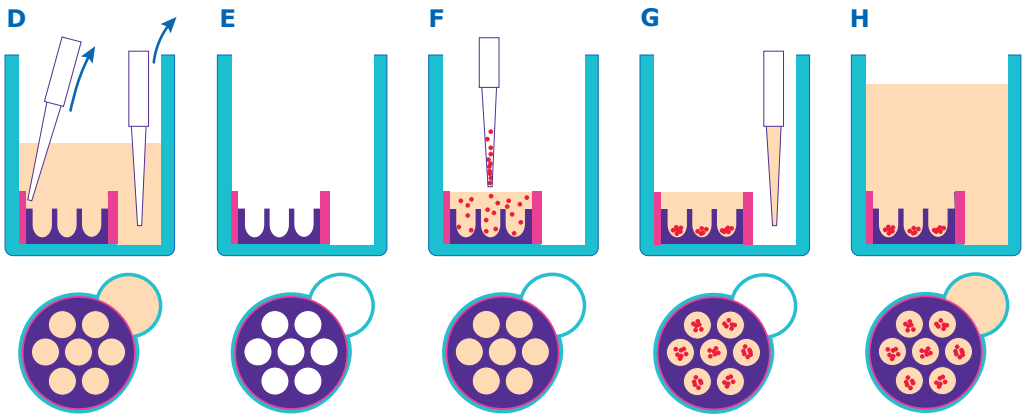
Cell Seeding Density

For 100 Cells Per Microwell

Microwell Diameter (µm)	400	600
Cell Seeding Density (Cells/mL)	2.42×10^5	1.10×10^5

Note: When working with highly proliferative cells such as stem cells, the recommended minimum number of cells per microwell is 100 cells. In non-proliferative cells such as PHH spheroids, we would recommend 500-1000 cells per microwell in the 400 µm size, 1000-2000 cells per microwell in the 600 µm size microwells.

Single Cells Seeding in the Microwells

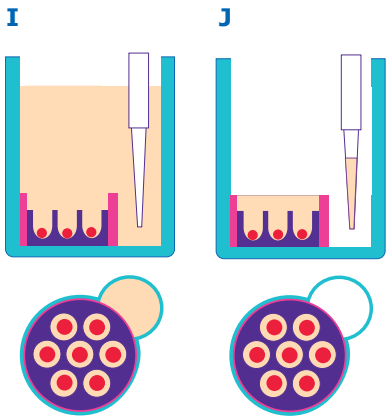


Remove medium from pipetting port and cell seeding chamber (D-E). Add 50 µL of cell suspension directly above the cell seeding chamber (F). Then add 150 µL of growth medium into the pipetting port (G-H).

5. Remove the medium from both the pipetting port and the cell seeding chamber with a wide-bore pipette tip connected to an aspirator as ishown above (D). Be very careful not to damage the hydrogel microwells.
6. Seed cells by pipetting 50 µL of the prepared single cell suspension directly from above of the cell seeding chamber of each microwell array (F).
   Caution: Avoid touching the hydrogel microwells.
7. Allows cells to sediment for at least 20-30 minutes in a 37 °C with 5% CO_2 incubator.
8. Remove plate from the incubator and carefully add 150 µL of growth medium slowly into the pipetting port (G-H).

   Note: if culture requires BME (Basement Membrane Extract) to grow, then dilute the BME to the desired final percentage, (typically 1.6 to 2.0%), into 150 µL of growth medium. The final percentage of BME should be for 200 µL volume (150 µL + 50 µL of cells). Afterward, slowly add into the pipetting port. An example of a BME used in 3D culture is Corning GFR Matrigel (356231).
9. Put plate back in a 37 °C with 5% CO_2 incubator.

3D Cell Culture Maintenance Using Millicell® Microwell Plates



10. Change medium to your 3D cultures every 2-3 days by aspirating medium from the pipetting port (I) and then slowly add back the 150 µL of growth medium into it (J).

    Note: For cultures that require BME to grow, add 1% BME in the medium at every medium changes. An example of a BME used in 3D culture is Corning GFR Matrigel (356231).

11. Put plate back in a 37 °C with 5% CO_2 incubator.

Product Ordering

Order online at SigmaAldrich.com.

Millicell® Microwell Plates are in a polystyrene 96-well format.

Description	# of Microwells per well	Pack	Catalogue Number
Millicell® Microwell 400 µm U-microwell 96-well Hydrogel Plates	121*	5 pack	MC96U4005
Millicell® Microwell 600 µm U-microwell 96-well Hydrogel Plates	55*	5 pack	MC96U6005

* Shown are maximum number of microwells per well. Actual number of microwells may be up to 7% fewer.

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