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Organoid Culture Protocol using Millicell® Microwell Plates

Hydrogel microwell arrays for reproducible long-term culture of organoids

Introduction

Millicell® Microwell plates are ideal to generate homogeneous organoid cultures. Each well contains a standardized, micro-structured hydrogel array of U-shaped microwells enabling 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 96-well plates exist with different microwell sizes, accommodating for initial aggregate size as well as the growth of the organoid over time.

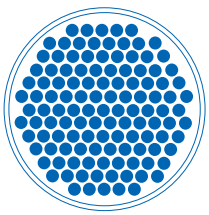
Methods

Millicell® Microwell 96-well plates should be stored in the refrigerator at 4-8°C away from direct sunlight. The plates are stable for a minimum of 6 months after arrival. The plates contain a buffer to keep the hydrogel array hydrated and are vacuum-sealed to avoid the buffer from leaking.

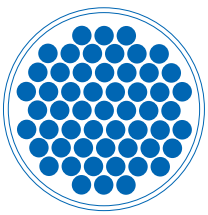
Microwell Sizes (µm)	Number of Microwells/ well	Working Volume (µl)	Bottom	Cat. No
400µm	121*	200	Plastic	**MC96U4005**
600µm	55*	200	Plastic	**MC96U6005**

*Indicates maximum possible number of microwells per well. Exact number may differ slightly from well to well, and may vary by 7% fewer.

400 µm



600 µm



A. Preparation of Millicell® Microwell 96-well plates

Before use, sterilize the plate with its outer plastic wrapping. Remove the wrapping using the helping notch while keeping the plate levelled. Open the plate lid, and remove the sealing layer inside the lid.

1. Aspirate the shipping buffer from the media-changing port.
2. Equilibrate the hydrogel arrays in growth medium before seeding cells by adding 150 µl via the media-changing port into each well of the well plate.
   a. Alternatively, for precious medium, aspirate the shipping buffer from the inner cell seeding ring and incubate with 50 µl of the medium.
3. Incubate the Millicell® Microwell plate for at least 30 minutes at room temperature or at 37° C.

B. Generation of Cell Aggregates and Culture of Organoids

4. Prepare a single cell suspension in your desired medium.
   a. Count cells using a cell counter.
   b. Refer to the tables on the back side of this protocol document to determine the cell seeding density. Alternatively, use the following formula for preparing the stock cell seeding solution:

$$\text{Cell seeding density } \left(\frac{\text{cells}}{\text{ml}}\right) = \#\text{ cells per aggregate} * \#\text{ of microwells} * 20$$

NOTE: *5 ml of cell seeding solution is required to fill an entire 96 well plate.*

Continuation of step 4b from front:

The indent seeding volume per hydrogel array is 50 µl.

Microwell Size (in mm)	Microwell Number per Well
400	121*
600	55*

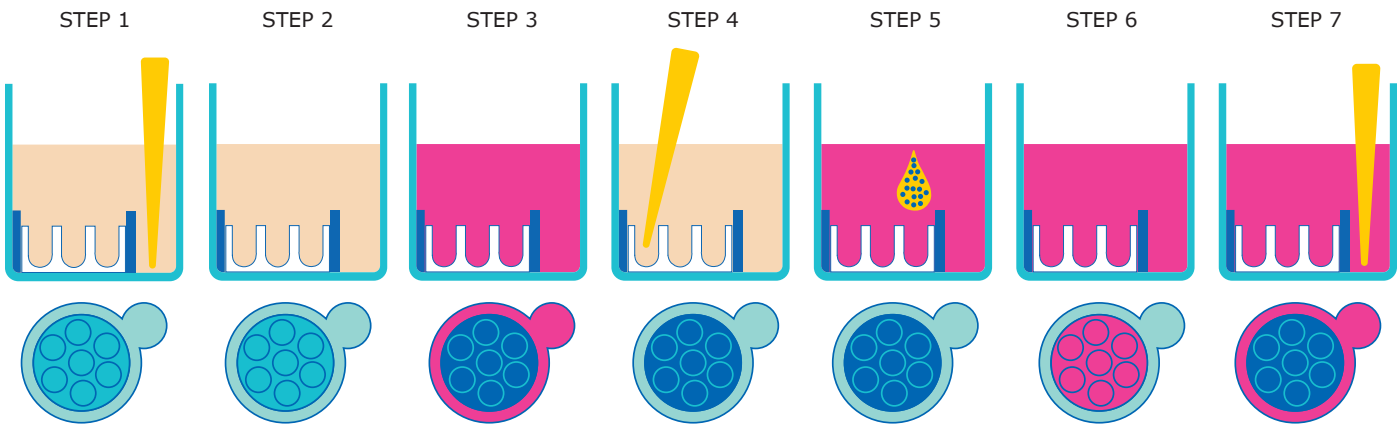
*Shown are maximum number of microwells.
Actual number may be up to 7% fewer.

Desired Number of Cells per Aggregate/Organoid	Cell Seeding Density (cells/ml for 1ml)*
100	$1.1x10e^5$
500	$5.5x10e^5$
1000	$1.1x10e^6$
3000	$3.3x10e^6$

*Cell densities are rounded up to next decimal

c. Remove the medium from the well and in the inner ring by carefully aspirating the medium. Be careful only to touch the top of the array at the interface of the ring to avoid damaging the hydrogel array.

5. Plate cells by pipetting 50 µl of the prepared cell seeding solution on top of the Millicell® Microwell plate array in each well. Incubate for a minimum 1 hour at 37°C.
6. Once cells have settled into the microwells, add complete growth medium at the desired working volume (typically add 150 µl to reach a working volume of 200 µl) slowly, via the media-changing port until the medium spills over the Millicell® Microwell plate array.
7. Continue with desired cell culture protocol, changing medium as desired by removing old medium as in **Step 1** and adding new medium as in **Steps 6-7**.



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