Organoid Culture Plate

Clear, non-treated plate for organoid cultures

Catalog #200-0561	5 x 24-Well Plate
Catalog #200-0562	5 x 96-Well Plate

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Product Description

The Organoid Culture Plate is a 3D culturing device that makes organoid expansion and differentiation easier, faster, and more robust. The plate ensures that matrix-based cultures are formed with controlled culture depth for more reproducible growth and remain centered in the wells for improved imaging.

- Prewarming is not required. Optimal performance is achieved in room temperature (15 - 25°C) plates.
- All wells in each plate can be used for organoid culture. Cultures need not be limited to the inner wells.
- Plates can be used with Corning® Matrigel® or with alternative matrices, including chemically, enzymatically, or photo-crosslinkable materials. The protocol should be adapted as necessary for gelation of alternative matrices.

These plates can be used in most organoid workflows traditionally cultured using dome culture, including IntestiCult™ Organoid Growth Medium (Human; Catalog #06010), IntestiCult™ Organoid Differentiation Medium (Human; Catalog #100-0214), and HepatiCult™ Organoid Growth Medium (Human; Catalog #100-0385).

Diagram

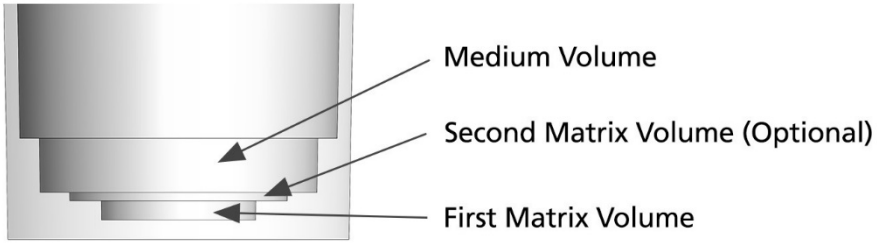

Figure 1. Overview of a single Organoid Culture Plate well

Each Organoid Culture Plate well contains features to define three distinct volumes. The First Matrix Volume can be used in place of the matrix dome used in traditional dome-based organoid culture methods. The optional Second Matrix Volume can be used to add a second layer of matrix and cells for layered co-culture. The Medium Volume can contain 500 µL of medium; however, medium can be added beyond the top of this feature if desired. The geometry of the features in each well ensures that all volumes are indexed to the center of each well to allow a standardized well position for seeding or imaging. Each volume maintains a controlled depth of the medium or matrix it contains.

Storage and Stability

Store Organoid Culture Plates at room temperature (15 - 25°C) away from direct sunlight. Stable for 5 years from date of manufacture (MFG) on label.

Directions for Use

NOTE: Do not expose Organoid Culture Plates to organic solvents, including ethanol or isopropanol.

To reduce biological variation, increase well-to-well consistency, and produce more uniform organoid size distributions, perform the optional steps in section A.

For culture plating in Organoid Culture Plates, substitute section B for relevant steps after the generation of a matrix/clump mixture in any suitable organoid workflow (e.g. section B, step 4 in Document #10000003510).

For medium addition and medium exchange using Organoid Culture Plates, refer to sections C and D.

A. (OPTIONAL) CLUMP SIZE RESTRICTION

1. Use sterile technique to prepare 2% bovine serum albumin (BSA) wash buffer. The following example is for preparing 50 mL of 2% BSA wash buffer. If preparing other volumes, adjust accordingly.
   a. Add 4 mL of 25% BSA to 46 mL of DMEM/F-12 with 15 mM HEPES (Catalog #36254) in a 50 mL conical tube (e.g. Catalog #38010).
   b. Mix well by inversion. Place on ice.

      NOTE: If not using immediately, store at 2 - 8°C for up to 6 months.
2. Filter the clump suspension prior to counting clumps.
   a. Place a 70 µm strainer (e.g. Catalog #27216) on top of a 15 mL conical tube and rinse with 1 mL Anti-Adherence Rinsing Solution (Catalog #07010), followed by 2 x 1 mL washes with 2% BSA wash buffer prepared in step 1.
   b. Filter fragments through the 70 µm reversible strainer into a new 15 mL conical tube.
   c. Wash reversible strainer with 1 mL of 2% BSA wash buffer.
   d. Centrifuge the conical tube at 100 x *g* for 5 minutes at 2 - 8°C. Gently pour off and discard the supernatant.
   e. Resuspend fragments in appropriate matrix, according to the passaging protocol for your organoid type.

B. PLATING

Up to two matrix layers of equal volumes can be cultured in each well of the Organoid Culture Plate. For standard organoid culture, only a single matrix volume is used.

1. Pipette the matrix/clump mixture to the center of the First Matrix Volume in one well. Use the following volumes of matrix/clump mixture, depending on the number of wells in your plate:
   - In a 24-well plate: Pipette 50 µL of matrix/clump mixture to the center of the First Matrix Volume
   - In a 96-well plate: Pipette 10 µL of matrix/clump mixture to the center of the First Matrix Volume
2. Inspect the well to ensure the matrix/clump mixture fills the volume fully, with no bubbles or gaps. If the mixture does not fill the volume fully, use the pipette tip to drag the mixture around the volume's edge.
3. Repeat steps 1 and 2 for all wells.
4. Allow the matrix to polymerize. For Matrigel®, incubate plates at 37°C for 10 minutes.
5. (OPTIONAL) Add a second matrix layer: Take plates out from the incubator, and repeat steps 1 - 4, beginning with pipetting an equal volume of matrix/clump mixture (used in section B step 1) to the center of the Second Matrix Volume.
6. Proceed to section C to add medium.

C. MEDIUM ADDITION

1. Add organoid growth medium at room temperature (15 - 25°C) near the outer edge of the well, taking care to avoid pipetting directly onto the matrix layer(s) below. Depending on your plate format and the number of matrix layers used, add the appropriate volume of medium as follows:
   - In a 24-well plate: If using one matrix layer, add 500 µL of medium. If using two matrix layers, add 450 µL of medium.
   - In a 96-well plate: If using one matrix layer, add 200 µL of medium. If using two matrix layers, add 190 µL of medium.
2. Incubate plates at 37°C for long-term culture.

D. MEDIUM EXCHANGE

1. Aspirate all medium from the bottom outer edge of the well to avoid disturbing the matrix layers.
2. To replace medium, follow the steps for medium addition (see section C).

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