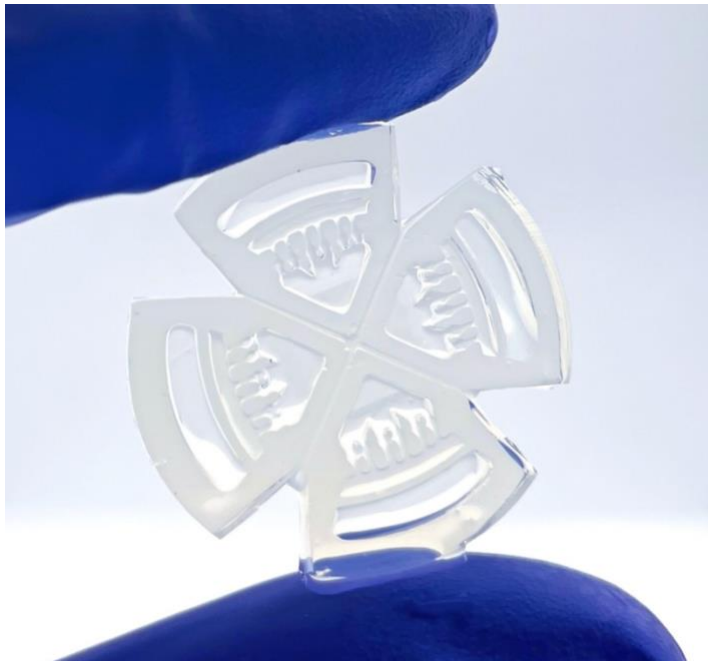




3-in-1 Plate

NEXT GEN TISSUE CULTURE



User Protocol

3-in-1 Plate User Guide

Materials Included

- Six transparent hydrogel inserts.
- One standard EPS six-well plate

Shipping & Storage

The inserts are shipped in 6 well plate and sealed sterile bags. The plates can be stored at 4°C temperature for up to 9 months from the date of production.

Important Starting Notes

1. The plates are shipped in a sterilized condition. **However, we recommend putting the plates under the UV light of the biosafety cabinet for 15 min before usage.**
2. To avoid contamination, only manipulate the plates and inserts using aseptic techniques in a laminar flow hood.
3. If condensation forms on the plates, gently wipe with an aseptic wiper.
4. Each insert is covered with a small amount of PBS and Pen-Strep. Aspirate all liquid from each insert before beginning your experiment.
5. When placing the plate in the incubator, gently add 500 μ L of PBS or culture media to the center of each **unused** insert to prevent it from drying out.
6. The inserts have been inserted at the bottom of each well for optimal imaging conditions. Removing the inserts and placing them in a new 6-well plate will reduce image quality.
7. Refer to the **3-in-1 Plate Data Sheet** for specific inquiries.
8. **This plate was optimized** for making tumor spheroids from various types of mesenchymal and epithelial cells. The protocol may need adjustments to satisfy your specific needs. Each insert contains four separate seeding compartments suitable for performing four different experimental conditions. The number of cells per seeding can be adjusted from 50K to 350K cells according to your desired spheroid size.

Seeding Protocol

1- Cell Seeding & Spheroid Formation

The number of cells seeded in each quadrant can range from 50K to 350K cells according to your desired spheroid size and the microwell diameter. Optimization of the number of cells seeded may be required. Each seeding produces six spheroids or organoids. This protocol is optimized for cancer cell spheroids

- 1-1- Using an aseptic technique in a laminar flow hood, open the sealed bag and add 25 μL of culture media in the Cell/ECM Loading Zone (Fig. 1) of each compartment of the insert.
- 1-2- After 2 minutes, add 50 μL of cell suspension¹ to the Cell/ECM Loading Zone of each compartment of the insert and incubate for 10 minutes under cell culture conditions to let the cells sediment into the microwells². Make sure to deposit the cell suspension close to the gel and drop-by-drop to avoid bubbles getting trapped. This process is illustrated in Fig. 2.
 - 1-2-1- **Optional Direct Co-culture:** any types of secondary cells present in the microenvironment can be prepared in a suspension with the primary cells and can be seeded in the Cell/ECM Loading Zone of each compartment.
- 1-3- After the cells have settled into the microwells, gently rinse any additional cells stuck in the channels of the Cell/ECM Loading Zone by adding 70-100 μL of pre-warmed medium to the Cell/ECM Loading Zone. Then, aspirate that media by placing the pipette tip as close to the microwells as possible. Remove any additional media from the Secondary Reservoir (see Fig. 1). **Repeat the rinsing step if you observe any remaining single cells in the channels of the Cell/ECM Loading Zone.**
- 1-4- Finally, add 80 μL of pre-warmed fresh culture media to the Media Reservoir and 80 μL to the Cell/ECM Loading Zone of each quadrant of the insert. Incubate under cell culture conditions.
- 1-5- Monitor spheroid formation and growth daily³ and change the media regularly⁴ until spheroids reach your desired size.

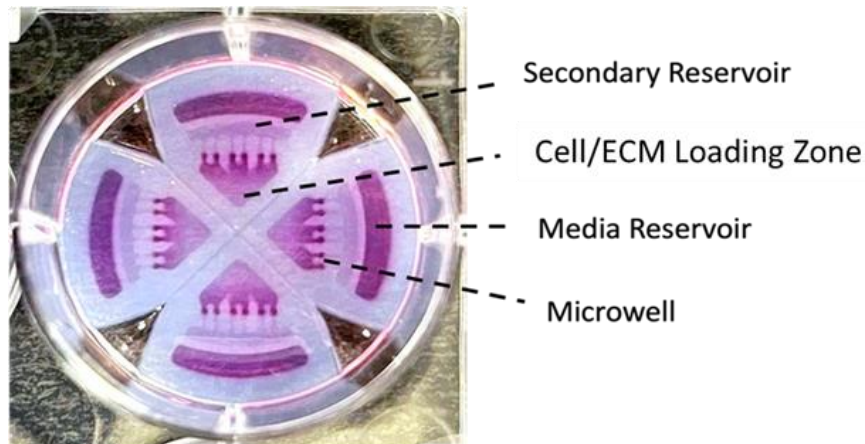


Fig. 1. Labeled Birds-Eye-View of the 3-in-1 Plate

¹ Prepare 50 μL of cell suspension to the desired concentration. The concentration used for a seeding number of 100K cell and 300K cells would be 2×10^6 and 6×10^6 cell /mL, respectively.

² This period of sedimentation may vary according to the cell type. For cells with smaller sizes, you need to incubate for a longer time.

³ Add fresh media before imaging for optimal image quality.

⁴ Refresh media as needed to ensure that media is always covering the spheroids. A variety of factors will affect the rate of media evaporation.

2- Extracellular Matrix Application

- 2-1- After spheroid formation inside the microwells of each quadrant of the insert, gently aspirate the media from the Media Reservoir⁵ and then add 40-60 μL of the desired hydrogel solution to the Cell/ECM Loading Zone⁶.
- 2-1-1 **Optional Co-culture:** any types of normal (stromal) cells present in the microenvironment can be prepared in a suspension with certain concentration and be mixed with the ECM hydrogel solution prior to applying it to the spheroids⁷.
- 2-1-2 **Secondary ECM Application:** if necessary, a secondary ECM can be added as an overlay to the primary ECM by adding it to the Secondary Reservoir (reservoir volume: 20 μL). Ensure that all media is aspirated before applying a secondary ECM.
- 2-1-3- **Immune Cell Co-culture:** immune cells can be mixed with a secondary ECM at a certain concentration and added to the Secondary Reservoir (Fig. 1)⁸.
- 2-2- After adding the ECM but before curing⁹, leave the plate for 2-5min (resting time)¹⁰ under the laminar flow hood to let the hydrogel solution fill the entire microchannel and microwells of each compartment.
- 2-3- After cross-linking the hydrogel, gently add 80 μL of pre-warmed fresh culture media to the Media Reservoir and 80 μL to the Cell/ECM Loading Zone of each compartment of the insert. Incubate under cell culture conditions.
- 2-4- Monitor spheroids daily and change the media regularly⁴ until spheroids reach your desired size.
- 2-5- For drug testing, simply mix your compound with media to the desired concentration and add to the Media Reservoir.

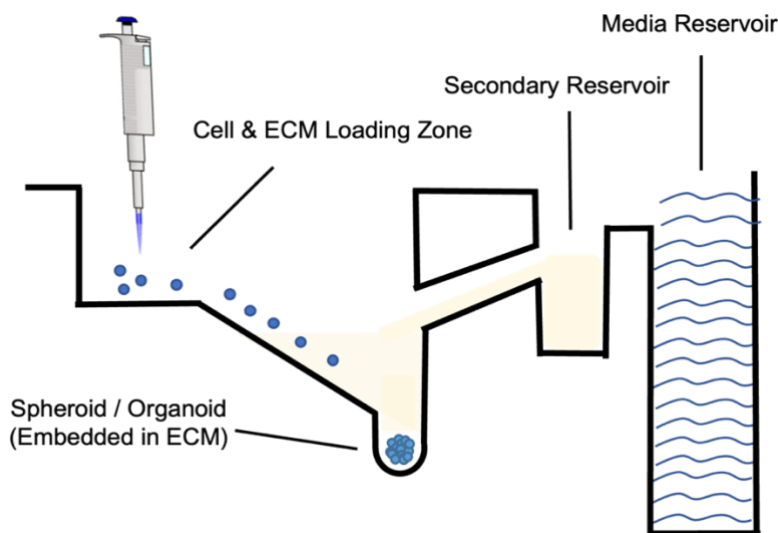


Fig. 2. Schematic cross-section of the 3-in-1 plate with location of Cell/ECM Loading Zone

⁵ After aspirating the media from the Media Reservoir, any additional media left in the microwells can be aspirated from the slope of the Cell/ECM Loading Zone.

⁶ The ECM Loading Zone is at the same location as the Cell Loading Zone. It is important to ensure that the hydrogel solution is not excessively viscous to the point that it cannot flow down the microchannels into the microwells.

⁷ In co-culture conditions, the concentration of the hydrogel solution should be adjusted with the cell suspension media.

⁸ Immune cells can also be mixed with media and added to the Media Reservoir.

⁹ Depending on the cross-linking method of the hydrogel, it can be cured using either temperature or light, etc.

¹⁰ This time may vary based on the viscosity of the hydrogel solution. Solutions with higher viscosity need more resting time.