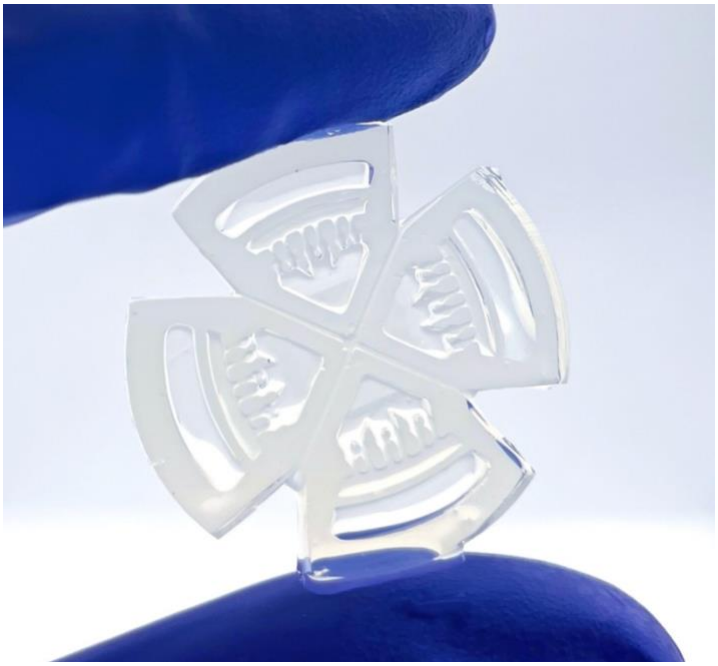
APRICELL BIOTECHNOLOGY

3-in-1 Plate

NEXT GEN TISSUE CULTURE



User Protocol

Immunofluorescence Staining

Materials Included

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

Shipping & storage

The inserts are shipped in a 6 well plate and sealed in sterile bags. The plates can be stored at 4°C 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 µL 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.

Immunostaining Protocol

1- Immunostaining of Tumoroids in the 3-in-1 Plate

- The 3-in-1 Plate is compatible with different imaging facilities including invert, up-right, fluorescent and confocal microscopes.
- For invasion monitoring within the ECM hydrogel, make sure to have sufficient media on top of the gel (in each quadrant of the insert). The insert is in a fixed position inside the 6 well-plate and is compatible with automated image acquisition systems.
- Fluorescent staining of cells is possible by on-plate staining of the spheroids.

This protocol is for general immunostaining of tumor spheroids.

1-1- Prepare 4% formaldehyde by diluting the stock 37% formaldehyde in PBS (for 10 mL, add 1.08 mL stock formaldehyde solution to 8.92 mL PBS). Aspirate the media from the Media Reservoir (Fig. 2) of the insert and add 200–300 µL of 4% solution to the reservoir and incubate at room temperature for 30 min[1].

1-2- Remove the formaldehyde solution and gently wash the tumoroids three times with PBS. Washing can be done by adding 200–300 µL of PBS to the Media Reservoir and incubating for 2 min to allow the materials to diffuse out of the gel into the washing solution.

1-3- Block the sample using BSA or normal serum related to the primary/secondary antibodies. Follow the procedure below to make the blocking buffer:

(a) Warm up PBS to 45–50 °C.

(b) Dissolve 0.3% wt/vol Triton X-100 in the warm PBS until it is clear.

(c) Weigh enough BSA to make a 5% (w/v) solution in a falcon tube. Wait until the Triton solution cools down to room temperature. Then add the 0.3% Triton solution to make the blocking buffer.

1-4- Aspirate the last wash solution from the Media Reservoir of the insert and add 200-300 µL the blocking buffer. Keep in the fridge overnight.

1-5- Make the primary antibody solution. Like the procedure for making the blocking buffer, make a solution of 1% (w/v) BSA and 0.3% (w/v) Triton in PBS. According to the protocol (for 3D culture use a decreased dilution factor), dilute the primary antibody in the 1% (w/v) BSA and 0.3% (w/v) Triton solution.

1-6- Aspirate the blocking buffer from the reservoir of the insert and add the primary antibody solution. 200 µL volume is enough and no washing step is required at this stage. Incubate overnight[2].

1-7- Remove the primary antibody solution from the Media Reservoir of the insert and wash it three

[1] Fixing reagent can be chosen by the user.

[2] Steps 7 and 8 are for non-conjugated primary antibodies. If the primary antibody is already conjugated go directly to step 9.

times according to step 2. Subheading 3-1-2.

1-8- Add secondary antibody solution. To make the solution of the secondary antibody, make 1% BSA and 0.3% Triton solution similar to step 5 and dilute the secondary antibodies in it according to the supplier's protocol (for 3D culture use a decreased dilution factor). Incubate for 4–6 h in the fridge.

1-9- Remove the solution of secondary antibodies and add the DAPI solution. To make DAPI solution dilute stock solution of DAPI in PBS according to supplier's protocol (for 3D culture use a decreased dilution factor). Incubate for 2–3 h at room temperature.

1-10- Remove the DAPI solution and wash the tumoroids three times with PBS. Then, add 300 µL of PBS to the wells and the tumoroids will be ready for immunofluorescence microscopy.

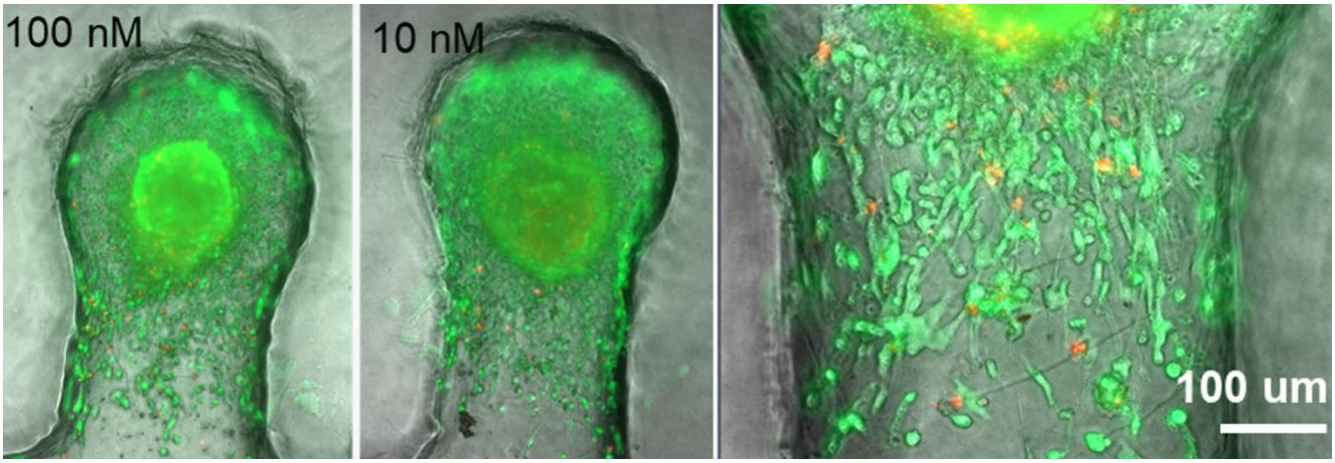


Fig. 1. Fluorescent images of the cancer cells invading into the ECM hydrogel and through the microchannels connected to the spheroid microwells.

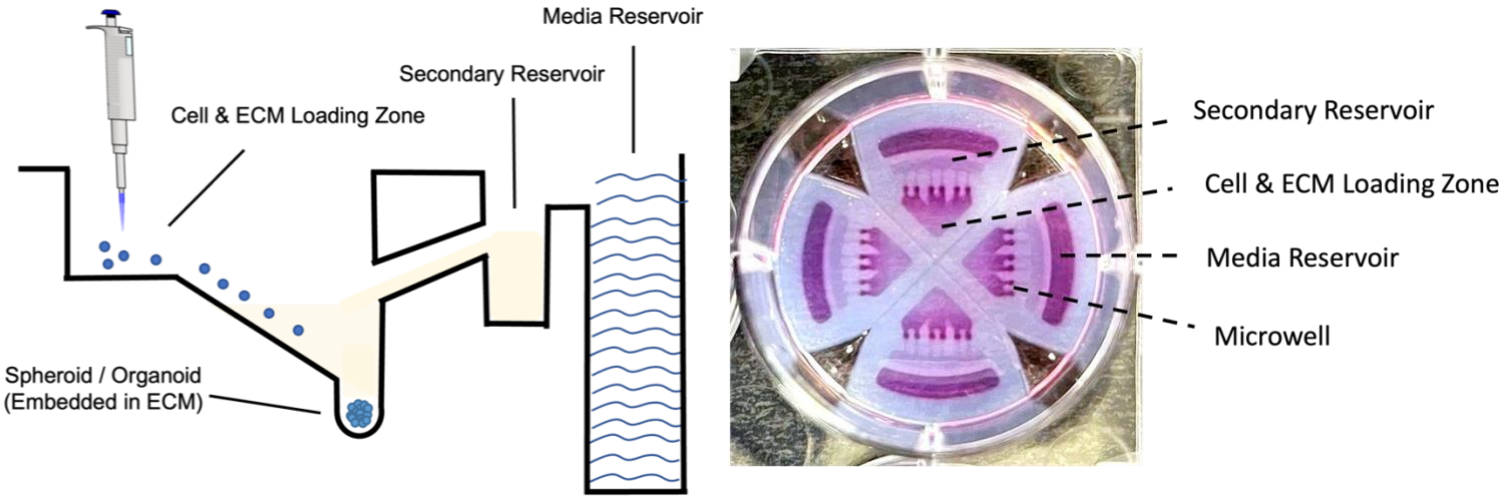


Fig. 2
Left image: side view schematic of one microwell of the 3-in-1 Plate.
Right image: birds-eye-view of a 3-in-1 Plate inside a well of a six-well plate.