TECHNICAL BULLETIN

Scale-Up and Scale-Down of Intestinal Organoids in Dilute Matrigel® Suspension Culture

Scaling intestinal organoid cultures does not have to rely on increasing plate numbers, Matrigel® domes, or hands-on manipulation. This protocol describes a static suspension culture method using dilute Matrigel® that enables both scale-up and scale-down of intestinal organoids beyond the limitations of traditional dome-based formats.

By maintaining organoids in a dilute Matrigel® suspension, this approach allows seamless adaptation across culture vessels ranging from small-well plates to larger flasks, while supporting robust growth and reproducibility. The format is particularly valuable for workflows that require increased cell numbers, higher throughput, or improved operational efficiency, such as large-scale expansion, screening-based studies, assay development in smaller well formats, and experimental designs where Matrigel® domes are not practical.

This protocol is optimized for use with IntestiCult™ Plus Organoid Growth Medium and provides a flexible, scalable solution for intestinal organoid culture.

Materials

- IntestiCult™ Plus Organoid Growth Medium (Catalog #100-1677)
- DMEM/F-12 with 15 mM HEPES (Catalog #36254)
- 25% bovine serum albumin (BSA) in phosphate-buffered saline (PBS)
- Gentle Cell Dissociation Reagent (GDCR; Catalog #100-0485)
- Anti-Adherence Rinsing Solution (Catalog #07010)
- Corning® Matrigel® Matrix, Growth Factor Reduced (GFR), Phenol Red-Free (Corning Catalog #356231)
- D-PBS (Without Ca++ and Mg++) (Catalog #37350)
- Falcon® Serological Pipettes, 5 mL or 10 mL (Catalog #38003 or 38004)
- Falcon® 96-Well Flat-Bottom Microplate, Tissue Culture-Treated (Catalog #38022)
- Costar® 24-Well Flat-Bottom Plate, Tissue Culture-Treated (Catalog #38017)
- Costar® 12-Well Flat-Bottom Plate, Tissue Culture-Treated (Catalog #200-0624)
- Costar® 6-Well Flat-Bottom Plate, Tissue Culture-Treated (Catalog #38015)
- Falcon® 25 cm² Rectangular Canted Neck Cell Culture Flask with Vented Cap (Corning Catalog #353109)
- Falcon® 75 cm² Rectangular Canted Neck Cell Culture Flask with Vented Cap (Catalog #200-0501)
- Organoid Culture Plate, 24 Wells (Catalog #200-0561)
- Organoid Culture Plate, 96 Wells (Catalog #200-0562)
- Conical Tubes, 15 mL and 50 mL (Catalog #38009 or #38010)
- Antibiotics (e.g. gentamicin or penicillin/streptomycin)

Preparation of Reagents

A. Thawing and Aliquoting IntestiCult™ Plus Organoid Growth Medium.

1. Thaw IntestiCult™ Plus Basal Medium at room temperature (15 - 25°C) or at 2 - 8°C overnight. Mix thoroughly.
2. Thaw IntestiCult™ Plus 1000X Supplement on ice.

Note: *We do not recommend thawing or pre-warming IntestiCult™ Plus at 37°C.*

Note: *Once thawed, use each component immediately or aliquot and store at -20°C for up to 12 months. After thawing the aliquots, use immediately or store for up to 3 weeks at 2 - 8°C. Do not re-freeze.*

B. Complete IntestiCult™ Plus Start Medium

Use sterile technique to prepare complete IntestiCult™ Plus **Start** Medium (Basal Medium + 1000X Supplement). The following example is for preparing 10 mL of Start Medium. If preparing other volumes, adjust accordingly.

1. Thaw IntestiCult™ Plus Basal Medium and IntestiCult™ Plus 1000X Supplement as directed in Preparation of Reagents, part A.
2. Add 50 µL of 1000X Supplement to 10 mL of IntestiCult™ Plus Basal Medium (final concentration 5X). Mix thoroughly.

   Note: *If not using immediately, store the complete medium at 2 - 8°C for up to 3 weeks.*

3. Add desired antibiotics immediately before use (e.g. 50 µg/mL gentamicin or 100 units [100 µg/mL] penicillin/streptomycin).

C. Complete IntestiCult™ Plus Balance Medium

Use sterile technique to prepare complete IntestiCult™ Plus **Balance** Medium (Basal Medium + 1000X Supplement). The following example is for preparing 10 mL of Balance Medium. If preparing other volumes, adjust accordingly.

1. Thaw IntestiCult™ Plus Basal Medium and IntestiCult™ Plus 1000X Supplement as directed in Preparation of Reagents, part A.
2. Add 10 µL of 1000X Supplement to 10 mL of IntestiCult™ Plus Basal Medium (final concentration 1X). Mix thoroughly.

   Note: *If not using immediately, store the complete medium at 2 - 8°C for up to 3 weeks.*

3. Add desired antibiotics immediately before use (e.g. 50 µg/mL gentamicin or 100 units [100 µg/mL] penicillin/streptomycin).

D. DMEM + 1% BSA

Use sterile technique to prepare DMEM + 1% BSA. The following example is for preparing 50 mL of DMEM + 1% BSA. If preparing other volumes, adjust accordingly.

1. Add 2 mL of 25% BSA to 48 mL of DMEM/F-12 with 15 mM HEPES in a 50 mL conical tube.
2. Mix well by inversion. Place on ice.

Note: *If not using immediately, store at 2 - 8°C for up to 6 months.*

E. Preparation of Suspension Medium

1. Thaw and store Matrigel® on ice.
2. Chill an appropriate volume of complete IntestiCult™ Plus Organoid Growth Medium (Start or Balance) on ice for at least 15 minutes using sterile technique.
3. Add an appropriate amount of Matrigel® (5% v/v) to cold IntestiCult™ Plus Start Medium, making sure to completely mix the solution. Continue to store on ice until needed.

F. Cultureware Preparation

1. If using 24-, 12-, or 6-well plates or flasks, coat with Anti-Adherence Rinsing Solution to avoid organoids adhering to the bottom.

   Note: *If using a 96-well plate or a 96- or 24-well organoid culture plate, do NOT coat with Anti-Adherence Rinsing Solution. You will want these organoids to gently adhere to the bottom of the well to facilitate media changes. Anti-Adherence Rinsing Solution can also be omitted when using larger plate formats to facilitate in-well media changes.*

2. Allow the cultureware to cool down by placing at 2 - 8°C for at least 10 minutes.

Table 1. Recommended Number of Fragments and Growth Medium Volumes for Various Culture Formats

Culture Format	Number of Fragments to Seed Per Well/ Flask	Approximate Volume of Growth Medium per Well/Flask
96-Well Plate	300 - 400	100 µL
96-Well Organoid Culture Plate	200 - 300	100 µL
24-Well plate	1,500 - 2,000	0.5 - 1 mL
24-Well Organoid Culture Plate	1,000 - 1,500	0.5 - 1 mL
12-Well Plate	3,000 - 4,000	1 - 2 mL
6-Well Plate	5,000 - 8,000	3 - 3.5 mL
T25 Flask	15,000 - 50,000	5 mL
T75 Flask	45,000 - 150,000	15 mL

Suspension Protocol

Note: *If passaging from Matrigel® dome cultures, please follow steps 5 -15 of Section C of the IntestiCult™ Plus Product Information Sheet for clump passaging or sections D and E of the IntestiCult™ Plus Product Information Sheet for single cell passaging and continue to step 8.*

If passaging from a suspension culture, proceed as follows:

1. Using a serological pipette, transfer the contents of all wells/flasks to be passaged to a 15 or 50 mL conical tube. Centrifuge at 300 x *g* for 5 minutes.

2. Remove and discard as much of the spent medium as possible without aspirating any organoids and add 1 mL (5 - 10 mL if using a T25 flask or larger) of GCDR to the tube.

3. Incubate the tubes upright at room temperature for 10 minutes.

4. Centrifuge the tubes at 300 x *g* for 5 minutes at 2 - 8°C. Discard the supernatant.

5. Add 1 mL of ice-cold DMEM + 1% BSA to each tube. Using a pre-wetted 1 mL pipette tip, resuspend and fragment the organoids by pipetting up and down vigorously 15 - 25 times.

    - Depending on the amount of organoids pooled into a single tube, top up to 10 mL with ice-cold DMEM + 1% BSA after the trituration step.

    - Organoid cultures with thicker epithelia will require more triturations than those with thinner epithelia.

    - Count fragments according to section B, step 15 of the IntestiCult™ Plus Product Information Sheet.

6. Add an appropriate volume of the organoid suspension, corresponding to the desired number of fragments (Refer to Table 1), to a new 15 mL conical tube.

7. Centrifuge the sample at 200 x *g* for 5 minutes. Remove and discard as much of the supernatant as possible.

8. Resuspend the pellet with an appropriate amount of IntestiCult™ Plus Start Medium (with Matrigel®) and transfer into appropriate cultureware.

    Note: *If using 96 or 24-well Organoid Culture Plates, resuspend the pellet into 10 or 50 µL of medium, respectively, and transfer to the middle of the inner step of a single well. After waiting ~5 minutes, top up to 100 or 500 µL of medium, respectively, by slowly adding to the side of the well. This is done to prevent organoids from settling outside of the "step" area of the well.*

9. Add sterile PBS to unused wells and incubate culture at 37°C and 5% CO_2.

10. Every 2 - 3 days, perform a full medium change with IntestiCult™ Plus Balance Medium

    - If the cultureware was coated with anti-adherence solution:

        i. Transfer organoid suspension culture to a 15 or 50 mL conical tube using a wide bore pipette tip or serological pipette (avoid fragmenting the organoids).

        ii. Centrifuge tube at 290 x *g* for 5 minutes and slowly discard the supernatant.

        iii. Resuspend the pellet with an appropriate volume of IntestiCult™ Plus Balance Medium (supplemented with 5% Matrigel®) and transfer to the original cultureware.

    - If cultureware was not coated with an anti-adherence solution (e.g. 96-well plate):

        i. Centrifuge plate at 290 x *g* for 5 minutes.

        ii. Aspirate media carefully without touching the bottom of the well and replace with an appropriate volume of IntestiCult™ Plus Balance Medium (supplemented with 5% Matrigel®).

Note: *Some organoids may remain loosely adherent to the bottom of the well or flask during the media change, even when coated with anti-adherence solution. This is not a problem and they can remain loosely attached when the media is replaced. Gentle agitation or incubation with GCDR can release the organoids for collection, if desired.*

11. Passage the cultures when organoids are fully matured (5 to 8 days).

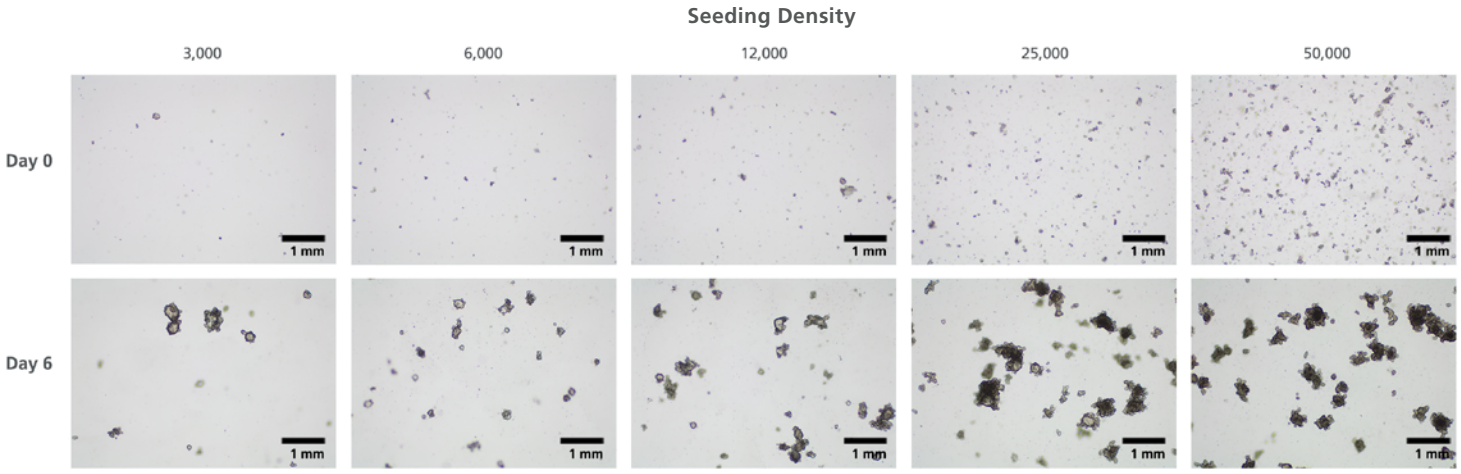

Figure 1. Morphology of Duodenum-Derived Intestinal Organoid Fragments

Morphology of duodenum-derived intestinal organoid fragments at various seeding densities, from 3,000 up to 50,000. Organoid fragments are shown at Day 0 and Day 6, cultured in 5 mL suspension of Matrigel® diluted with IntestiCult™ Plus. Scale bar = 1 mm.

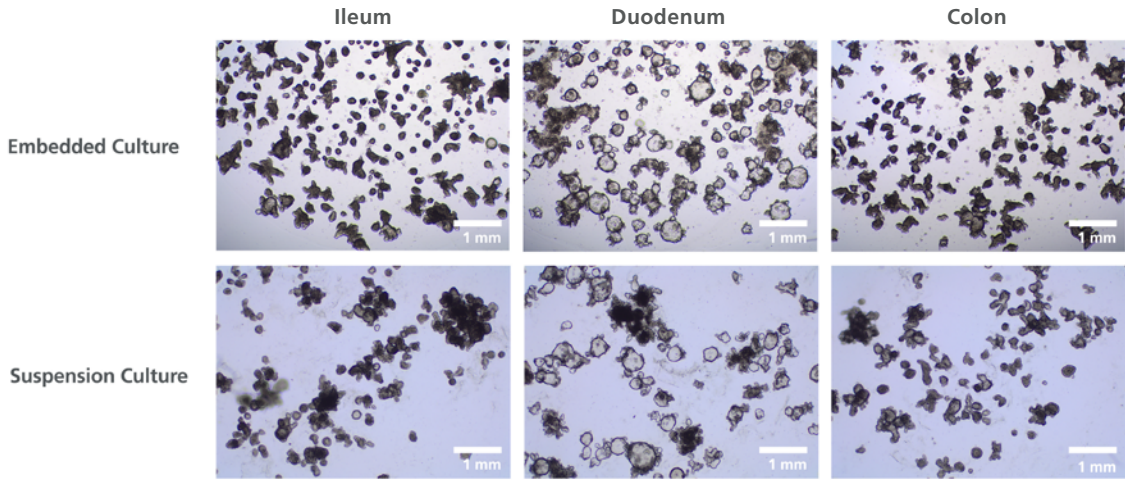

Figure 2. Morphology of Intestinal Organoids Cultured Using Either Embedded Culture or Suspension Culture

Morphology of intestinal organoids derived from ileum, duodenal, and colonic tissue cultured using IntestiCult™ Plus using either embedded (dome) culture, or dilute matrigel suspension culture on Day 7. Scale bar = 1 mm.