# Culture Mouse Intestinal Organoids in **Vivogel ® Matrix For Organoid Culture**

Application Note

Catalog #: VM004-10, VM004-PRF-10

Package size: 10 mL

## Application Introduction:

Organoids are simplified three-dimensional (3D) organ mimics which constitute next generation *in vitro* medical research models for applications in the fields of basic biology, disease modeling, drug discovery, and regenerative medicine. Self-organization of stem cells and progenitor cells, as well as their differentiated offspring, renders formation of organoids. Thanks to the rapid self-renewing ability inherited from intestinal epithelium, intestinal organoid is the most prevalent organoid kind that permits robust *in vitro* therapeutic research. The stem cells located at the bottom of the intestinal crypts are able to renew and differentiate into types of cells including enterocytes, goblet cells, paneth cells, and enteroendocrine cells that are essential for development of intestinal organoids. Successful cultivation of organoids *in vitro* requires 3D matrix that precisely recapitulates the physical properties and biochemical compositions of the naïve extracellular matrix environment.

**Vivogel ® Matrix** is a solubilized basement membrane extracted from the Engelbreth-Holm-Swarm (EHS) mouse tumor that comprises extracellular matrix proteins including laminin (glycoprotein), collagen IV, nidogen (glycoprotein), perlecan (heparan sulfate proteoglycan), and many other essential growth factors. **Vivogel ® Matrix** has empowered applications such as stem cell culture, angiogenesis assays, and tissue engineering. **Vivogel ® Matrix For Organoid Culture** is an optimized product specifically developed to support organoid growth and differentiation. Each lot of **Vivogel ® Matrix For Organoid Culture** is qualified to form stable 3D domes commonly used in organoid culture protocols.

This note demonstrates that **Vivogel ® Matrix For Organoid Culture** has the ability to sustain culture of mouse intestinal organoids for more than seven passages in IntestiCult ™ Organoid Growth Medium (Mouse) (STEMCELL Technologies). The organoids showed typical budding morphology with the differentiated intestinal cells expressing specific surface markers.

## Product specifications:

Concentration: 8 - 12 mg/mL.

Source: Murine Engelbreth-Holm-Swarm (EHS) tumor.

Buffer: DMEM (with phenol red) / DMEM (Phenol red free, PRF), with 10 μg/mL gentamicin.

Storage: -80 ℃ for long-term storage. Do not use **Vivogel ® Matrix** that has been stored at 4 ℃ for more than 24 h. Please aliquot upon receival of the product. Avoid multiple freeze-thaw cycles.

## Procedure guidelines:

**A.a.** GENERAL HANDLING OF **Vivogel ® Matrix For Organoid Culture**

Aliquots of **Vivogel ® Matrix For Organoid Culture** are thawed as needed from -20/-80 ℃. All steps involving **Vivogel ®** are to be finished on ice and require uses of pre-chilled tips and tubes. For organoid revival and culture, it is most recommended to submerge an aliquot of **Vivogel ®** in an insulated ice bucket in 4 ℃ overnight to enable mild thawing.

**A.b.** MATERIALS AND REAGENTS

| PRODUCT NAME | SUPPLIER | CATALOG # |
|---|---|---|
| IntestiCult Organoid™ Growth Medium (Mouse) | STEMCELL Technologies | 06005 |
| **Vivogel ® Organoid** | **Vivomatter Biotech** | VM003 |
| Gentle cell dissociation reagent | STEMCELL Technologies | 100-0485 |
| DMEM/F- 12, HEPES | Thermo Fisher Scientific | 11330032 |
| Bovine serum albumin (BSA) | Thermo Fisher Scientific | B14 |
| Gentamicin | Thermo Fisher Scientific | 15750060 |
| DPBS | | |
| Tissue culture-treated culturewares (multi-well plate, flask) | | |
| 50/15 mL conical tubes | | |

**B.a.** ACQUIRING MOUSE INTESTINAL CRYPTS FROM FRESH SMALL INTESTINE COLLECTIONS

1. Thaw 1 vial of **Vivogel ® Organoid** on ice.

2. Warm a tissue culture-treated 24 - well plate in a 37 ℃ incubator for at least 30 minutes.

3. Sacrifice mouse according to approved institutional guidelines. Harvest approximately 20 cm of small intestine.

4. Place the intestine in a 10 cm petri dish containing 5 mL of cold (2 – 8 ℃) Dulbecco's phosphate buffered saline (DPBS).

5. Flush the intestine gently with 1 mL of cold (2 – 8 ℃) DPBS by inserting a 1 mL pipette tip into one of the open ends of the intestine.

6. Use surgical scissors to make a longitudinal incision along the entire length of the intestine. Splay open the intestinal segment and use a pipette to wash gently with 1 mL of cold (2 – 8 ℃) DPBS for three times.

7. Transfer the intestinal segment to a clean 10 cm dish containing 15 mL of fresh cold (2 – 8 ℃) DPBS. Using forceps, move the segment through the clean buffer to rinse thoroughly.

8. Add 15 mL of cold (2 – 8 ℃) DPBS to a 50 mL conical tube. Using forceps, hold the washed intestine by one end over the tube. Starting from the bottom of the intestine, use scissors to cut the intestine into 2 mm segments, allowing these segments to fall into the conical tube.

9. Use a pre-wetted 10 mL serological pipette to wash the intestinal pieces by pipetting up and down 3 times. Let intestinal pieces settle by gravity and carefully remove supernatant. Add 15 mL of cold (2 – 8 ℃) DPBS. Repeat this wash procedure 15 - 20 times or until supernatant is clear.

10. Remove supernatant and resuspend the intestinal segments in 25 mL of Gentle Cell Dissociation Reagent at room temperature (15 – 25 ℃). Incubate on a rocking platform at 20 rpm for 15 min at room temperature (15 – 25 ℃).

11. Let the intestinal pieces settle by gravity for approximately 30 seconds and carefully remove the supernatant.

12. Resuspend the intestinal pieces in 10 mL of cold (2 – 8 ℃) DPBS + 0.1% BSA and pipette up and down 3 times.

13. Let the majority of the intestinal pieces settle to the bottom. Remove the supernatant and pass it through a 70 µm strainer into a 50 mL conical tube. Label the filtrate "Fraction 1" and place on ice.

14. Repeat steps 12 - 13 three times to generate Fractions 2 - 4.

15. Centrifuge the fractions at 290 × *g* for 5 minutes at 2 – 8 ℃. Carefully pour off and discard the supernatants.

16. Resuspend each pellet in 10 mL of cold (2 – 8 ℃) DPBS + 0.1% BSA.

17. Transfer each suspension to a fresh 15 mL conical tube labeled with the corresponding fraction number.

18. Centrifuge the fractions at 200 × *g* for 3 minutes at 2 – 8 ℃. Gently pour off and discard the supernatants.

19. Resuspend each crypt pellet in 10 mL cold (2 – 8 ℃) DMEM/F- 12 with 15 mM HEPES.

20. Add 1 mL of each suspension to individual wells of a 6 - well plate and assess the quality of the suspensions by using an inverted microscope. Select the suspensions that are enriched for intestinal crypts.

*NOTE: Crypts desirable for culture can be of various sizes and resemble small, folded sections of an epithelial monolayer. Fractions 3 and 4 usually have the greatest enrichment for desirable crypts.*

21. For each selected fraction, count the number of crypts in a 10 µL aliquot using an inverted microscope. Calculate the number of crypts per mL of each fraction.

22. From the selected fraction, aliquot the crypts into a 15 mL conical tubes in the volume containing approximately 2000 crypts.

Refer to Section **C** for the following steps of intestinal organoid culture.

**B.b.** ACQUIRING MOUSE INTESTINAL ORGANOIDS FROM A CYROVIAL

1. Thaw 1 vial of **Vivogel ® Organoid** on ice.

2. Warm a 24 - well tissue culture-treated plate in a 37 ℃ incubator for 30 minutes.

3. Thaw cryovial containing organoids in a 37 ℃ water bath. It usually takes less than 3 minutes until the freezing medium becomes liquid completely and organoids are visible. Proceed to the following steps immediately.

4. Transfer the contents of the cryovial to a 15 mL conical tube and add 9 mL of DMEM/F-12 with 15 mM HEPES + 1% BSA. Gently pipette up and down 5 times using a serological pipette to resuspend organoids.

5. Count the number of organoids using an inverted microscope. Calculate the number of organoids per mL of the resuspension and aliquot the organoids into another 15 mL conical tube in the volume containing approximately 2000 organoids.

Refer to Section **C** for the following steps of intestinal organoid culture.

**C**. MOUSE INTESTINAL ORGANOID CULTURE

1. Centrifuge the organoid aliquots acquired from **B.a** or **B.b** at 200 × *g* for 5 minutes at 2 – 8 ℃. Remove and discard the supernatant.

2. Add 150 µL of complete IntestiCult ™ Organoid Growth Medium (Mouse) at room temperature to the organoid pellet.

3. Add 150 µL cold **Vivogel® Organoid** and pipette up and down gently 10 times to resuspend the pellet. Avoid introducing bubbles.

4. Carefully pipette 50 µL of the suspension into each of 5 wells of the pre-warmed 24-well plate. Hold the pipette tip just above the bottom of the plate and slowly dispense to the first stop of the pipette. The samples should form domes in the middle of each well. Avoid introducing bubbles.

5. Transfer the plate to a 37 ℃ incubator for 10 - 15 minutes until the **Vivogel® Organoid** is solidified. Do not disturb the domes during transfer.

6. Add 750 µL of complete IntestiCult ™ Organoid Growth Medium (Mouse) at room temperature (15 – 25 ℃) to each well by pipetting the medium gently down the wall of the well. Do not dispense directly onto the domed cultures.

7. Add sterile DPBS to any unused wells.

8. Place the lid on the culture plate and incubate at 37 ℃ with 5% $CO_2$.

9. Replace the culture medium every 3 days with 750 µL of fresh, complete IntestiCult ™ Organoid Growth Medium (Mouse) at room temperature (15 – 25 ℃). Observe the development of organoid with Nikon C2 confocal microscope (Figure 1). Mouse intestinal organoids should be passaged every 7 - 9 days with an average split ratio of ~1:5.

*NOTE: Crypts from the freshly collected small intestine typically start to bud after 2 - 4 days in culture.*

*For organoids revived from cryovial, passage organoids 2 times before downstream experiments for best result. Organoid growth will be slow at first, with spheroids forming within 1 - 2 days and budding after 5 - 7 days. Organoids should be ready for passaging after 5 - 7 days. After1 - 2 passages, typical organoid growth characteristics should be restored.*

**D.** MOUSE INTESTINAL ORGANOIDS PASSAGE

1. Thaw 1 vial of **Vivogel® Organoid** on ice.

2. Warm IntestiCult ™ Organoid Growth Medium (Mouse) to room temperature (15 – 25 ℃). Keep DMEM/F-12 with 15mM HEPES on ice.

3. Warm a tissue culture-treated 24 - well plate in a 37 ℃ incubator for at least 30 min.

4. Gently remove the medium from each well. Do not disturb the dome of **Vivogel® Organoid**.

5. Add 1 mL of Gentle Cell Dissociation Reagent on top of the exposed dome in each well. Incubate at room temperature (15 – 25 ℃) for 1 minute.

6. Using a pre-wetted 1000 µL pipette tip, pipette the solution in the well up and down approximately 20 times to disrupt the dome and break up the organoids.

7. Transfer the suspension to a 15 mL conical tube. Rinse the well with an additional 1 mL of Gentle Cell Dissociation Reagent and add to the same 15 mL conical tube.

8. Repeat steps 6 - 7 for each well to be passaged.

9. Incubate the 15 mL tubes containing the organoids on a rocking platform set at 20 rpm at room temperature (15 – 25 ℃) for 10 minutes.

10. Centrifuge the tubes at 290 × *g* for 5 minutes at 2 – 8 ℃. Gently pour off and discard the supernatant.

11. Using a pre-wetted serological pipette, resuspend the pellets in 10 mL of cold (2 – 8 ℃) DMEM/F-12.

12. Centrifuge at 200 × *g* for 5 minutes at 2 – 8 ℃. Gently pipette off and discard the supernatant.

Refer to Section **C** for the following culture steps.

**E.a.** WHOLE MOUNT IMMUNOFLUORESCENT STAINING OF MOUSE INTESTINAL ORGANOIDS

1. Remove the medium and wash **Vivogel® Organoid** containing mouse intestinal organoids with DPBS three times.

2. Fix organoids in 4% PFA for 1h at room temperature.

3. Wash organoids twice in DPBS for at least 15 min.

4. Permeabilize 3D cell models by washing them through a gradient of methanol at room temperature. Wash organoids twice in DPBS, once in 50% methanol in DPBS, once in 80% methanol in deionized water, and finally once in 100% dry methanol.

5. Wash the organoids once in 20% DMSO/methanol, once in 80% methanol in deionized water, once in 50% methanol in DPBS, once in 100% DPBS, and finally in DPBS with 0.2% Triton X-100.

6. Incubate samples with 1% BSA in 0.1% DPBS-tween for 1.5 h at 37 ℃ to block non-specific protein-protein interactions.

7. Incubate with primary antibody (E-cadherin (Abcam)) overnight at 4 ℃.

8. Incubate with secondary antibody, phalloidin (Thermo Fisher Scientific) and DAPI (Thermo Fisher Scientific) for 3 h with aluminum foil covered.

9. Wash samples with DPBS for 3 times and subject to confocal imaging (Leica TCS SP8).

**E.b.** IMMUNOCYTOCHEMISTRY (ICC) STAINING OF MOUSE INTESTINAL ORGANOIDS

1. Remove the medium and wash **Vivogel® Organoid** containing mouse intestinal organoids three times with DPBS.

2. Fix organoids in paraformaldehyde (4% in DPBS, Sigma-Aldrich) for 1 hour at room temperature.

3. Wash wells with DPBS three times.

4. Add DPBS containing bovine serum albumin (1%, Sigma-Aldrich) to the wells and disrupt **Vivogel® Organoid** domes by pipetting up and down. Transfer the organoid and organoid fragments to a 1.5 mL eppendorf tube.

5. Allow organoids to settle for a few minutes, or centrifuge 5 to 10 sec to speed up the process. Gently discard the supernatant without disturbing the organoids.

6. Add 30% sucrose solution to dehydrate the organoid sample overnight.

7. Embed the dehydrated organoids into OCT compound (Tissue-Tek) at -80 ℃.

8. Cryosection the organoid sample at 8 μm.

9. Block and permeabilize the samples with 0.1% Triton X-100 in 4% normal goat serum (Thermo Fisher Scientific).

10. Incubate the samples with 6 kinds of primary antibody [Vimentin Cytoskeleton (Abcam), Lysozyme (Abcam), chromogranin-A (Abcam), Mucin2 (Abcam), Villin (Santa Cruz Biotechnology)] overnight.

11. Incubate with corresponding secondary antibody for 3 h with aluminum foil covered.

12. Wash samples with DPBS for 3 times and proceed to confocal imaging (Leica TCS SP8). (Figure 2)

## Representative results:

Culture and passage of mouse intestinal organoids are tested in **Vivogel® Organoid** following the protocols above. Freshly harvested intestinal crypts that are cultured in **Vivogel® Organoid** successfully develop into organoids with the typical budding and lumen morphology (Figure 1A). The organoids are maintained in three batches of **Vivogel® Organoid** for up to seven passages (Figure 1B).

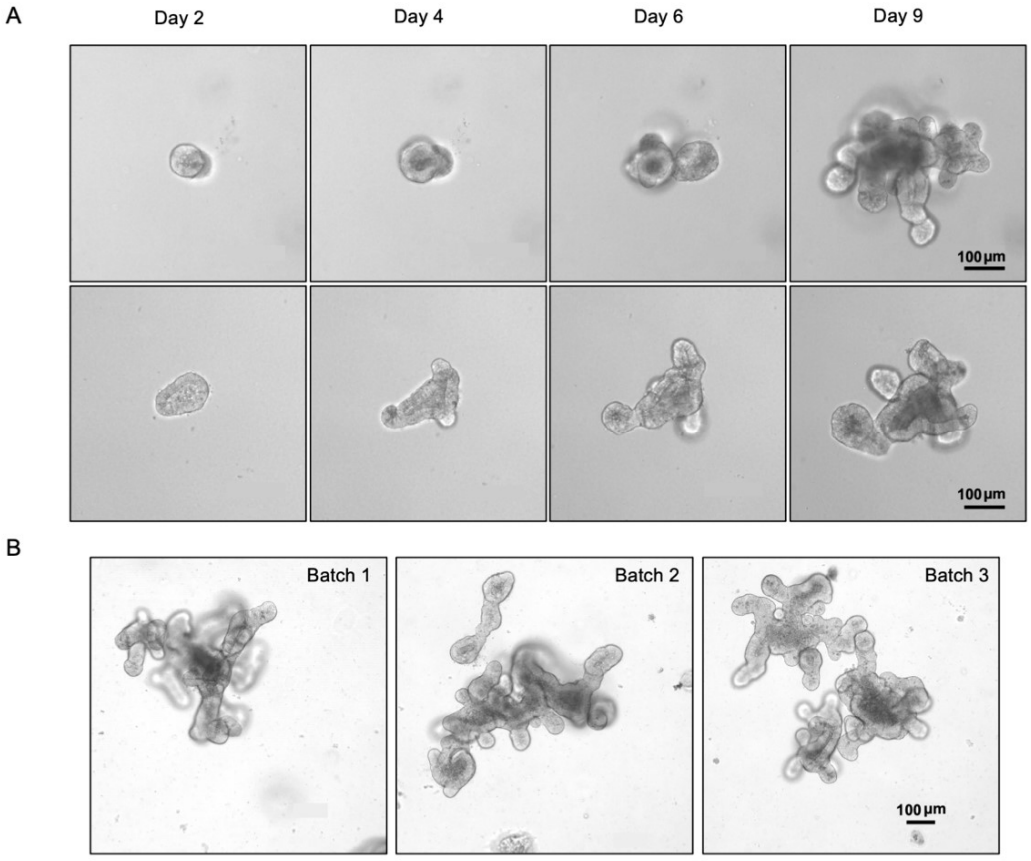


**Figure 1**. Mouse intestinal organoids cultured in **Vivogel ® Organoid**. (A) Representative images of primary culture of intestinal crypts freshly isolated from mouse small intestines in **Vivogel ® Organoid**. (B) Representative images of organoids cultured in three batches of **Vivogel ® Organoid** (Batch 1: passage 6; Batch 2: passage 10; Batch 3: passage 9).

Organoids can also be characterized by visualizing the overall organoid organizations (nuclei or F-actin) (Figure 2) or distributions of differentiated cell types, including intestinal mesenchymal cells, paneth cells, enterocytes, enteroendocrine cells, etc.) through immunocytochemistry (ICC) staining of specific biomarkers (Figure 3).

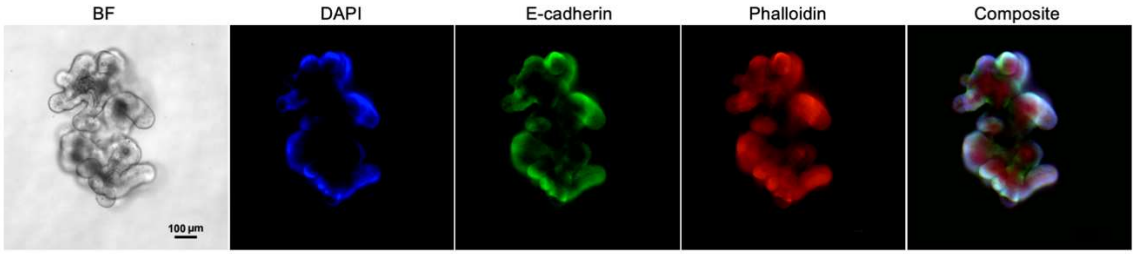


**Figure 2**. Representative immunofluorescent images of whole mount mouse intestinal organoids cultured in **Vivogel ® Organoid**. Organoids are stained with DAPI, E-cadherin and Phalloidin.

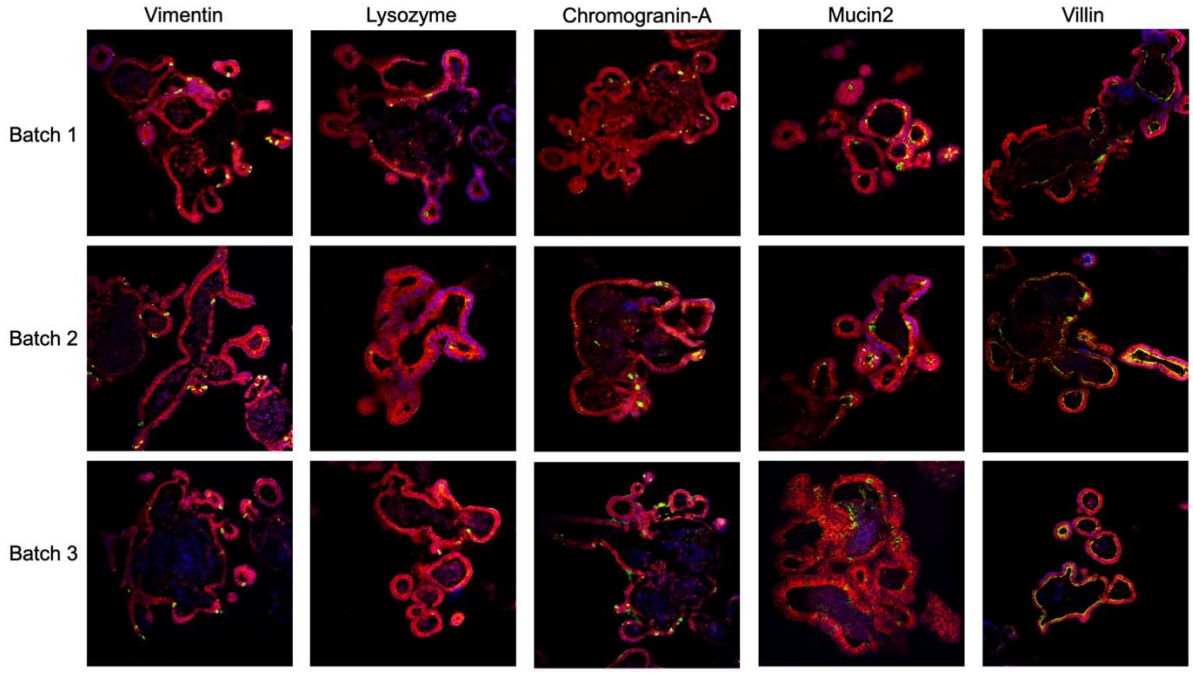


**Figure 3**. Representative immunofluorescent images of mouse intestinal organoids from three different batches (Batch 1: passage 6; Batch 2: passage 10; Batch 3: passage 9) in **Vivogel ® Organoid**. Distribution of differentiated intestinal cells, including mesenchymal cells, paneth cells, enteroendocrine cells, goblet cells and enterocytes in organoids are presented by biomarkers — vimentin, lysozyme, chromogranin-A, mucin 2 and villin, respectively. Blue: DAPI, Red: E-cadherin, and Green: specific marker.