In vitro Spheroid Formation Using Rapid & Round ™ Ultra-Low Adhesion Plate Vivogel ™ Matrix

Application Note

Catalog #: VM001-10, VM201-96R

Application Introduction:

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.

The VivoMatter ™ Rapid & Round ™ Ultra-Low Adhesion Microplate is a specialized laboratory tool designed for various applications, particularly in cell culture and high-throughput screening. **Rapid & Round ™ Ultra-Low Adhesion Technology** is a proprietary surface treatment by VivoMatter Biotech. The treatment is designed to reduce cell adhesion to the culture surface. In this note, we will use the plate for spheroid formation and culture.

Procedure guidelines:

A.a. GENERAL HANDLING OF **Vivogel ™ Matrix**

Aliquots of **Vivogel ™ Matrix** are thawed as needed from -20/-80 ℃. All steps involving **Vivogel ™ Matrix** are to be finished on ice and require uses of pre-chilled tips and tubes. Freeze thaws should be minimized by aliquoting into one time use aliquots. It is extremely important that **Vivogel ™ Matrix** and all cultureware or media coming in contact with **Vivogel ™ Matrix** should be pre-chilled/ice-cold since **Vivogel ™ Matrix** will start to gel above 10 ℃.

A.b. KEY MATERIALS AND REAGENTS

PRODUCT NAME	SUPPLIER	CATALOG #
Vivogel ™ Matrix	**Vivomatter Biotech**	VM001-10
Rapid & Round ™ Ultra-Low Adhesion Plate, 96-well, Round Bottom	**Vivomatter Biotech**	VM201-96R
MDA-MB-231 cells	ATCC	HTB-26

B. Procedures

1. **Seed MDA-MB-231 cells and allow them to grow.** Plate cells in an appropriate TC-treated culture vessel using complete DMEM. Allow the cells to reach 70 – 90% confluency which would usually take 3~4 days.

2. **Thawing ECM.** One day before the experiment, remove Vivogel from the freezer and place it in a refrigerator on ice. Thawing process will be completed after overnight-incubation at 4 °C.

3. **Cell Preparation.** On the day of experiment, medium from the vessel was aspirated, the cells were washed once in 1×PBS and dissociated using trypsin solution. The trypsin solution is then neutralized with complete DMEM.

4. **Cell seeding.** Cells are pelleted by centrifugation and then resuspended with complete DMEM containing 3% **Vivogel ™ Matrix** to 1×10^4 cells/mL. Aliquot 200 µL to each well of the Rapid & Round ™ plate, and place the plate in the incubator. You may alter cell density and volume depend on your application.

5. Spheroids will be ready on Day 4 (>2,000 cells) to Day 9 (<2,000 cells) depending on the seeded cell number.

Representative results:

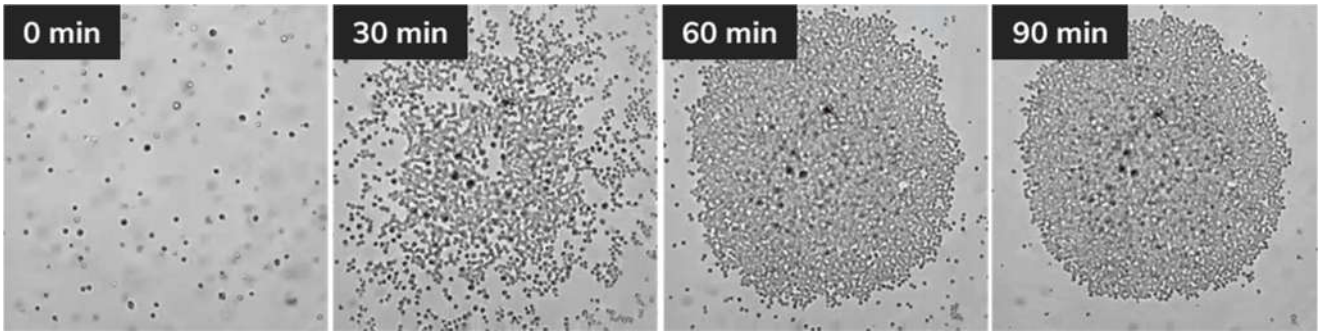

Figure 1. Upon added into the wells, cells quickly gathered at the bottom center of the wells.

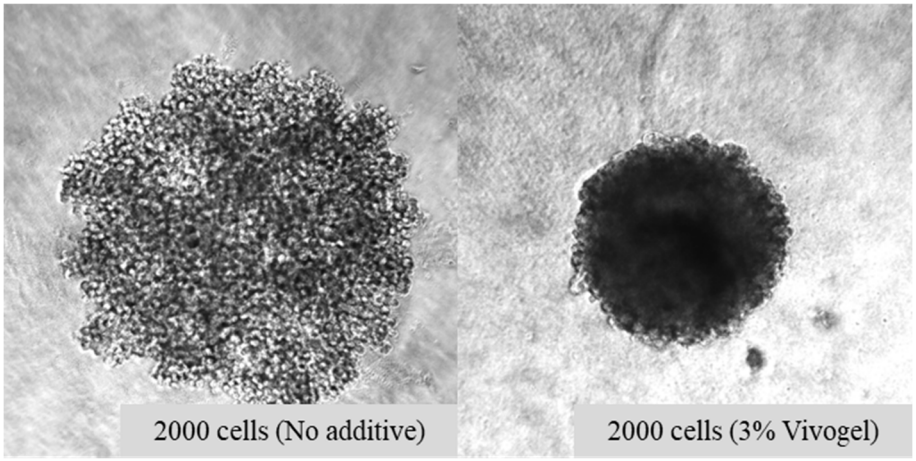

Figure 2. Typical morphology of spheroids on day 5.