Application Note

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

Catalog # VM001-10, VM201-96R

Application Introduction:

Spheroids are a classic and widely used model in the field of 3D cell culture, referring to spherical microtissues formed by cells through self-assembly. Compared to traditional 2D cultures, spheroids more accurately simulate the three-dimensional microenvironment in vivo, reflecting close cell-cell interactions and gradient distributions. As early as the 1970s, researchers began using techniques such as the hanging drop method to generate spheroids for tumor biology research. With continuous advancements in 3D culture technologies, spheroids have become ideal models for studying tumor invasion, drug screening, and stem cell differentiation.

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.

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. In this experiment, **Vivogel™ Matrix** helps cells to form more compact and stable spheroids, but the use of it is not mandatory in many cases. We strongly suggest you to conduct preliminary tests to learn about the spheroid-forming capability of your cells.

Procedure guidelines:

A.a. Key materials and reagents

PRODUCT	SUPPLIER	CATALOG No.
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

A.b. 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 ℃.

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 to Day 9.

Representative results:

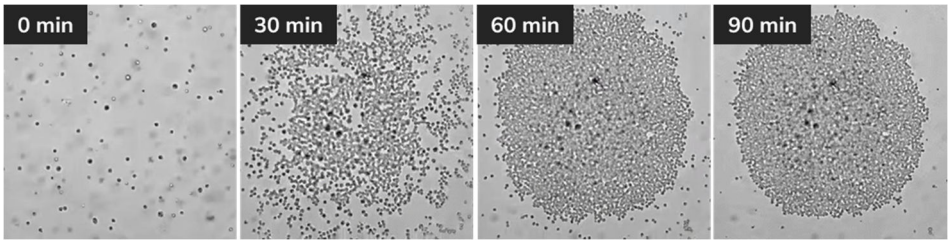

Figure 1. Upon added into the wells, cells quickly gathered at the bottom center of the wells.

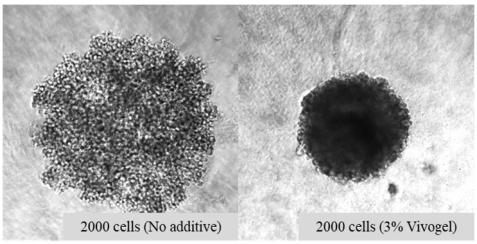

Figure 2. Typical morphology of spheroids on day 5.

Q&A

1. **Why use Rapid & Round™ Ultra-low-adhesion U-bottom plates for spheroid generation?**

   Ultra-low-adhesion plates prevent cell attachment to the plate surface, promoting cell-to-cell interactions. The U-bottom shape further encourages cells to settle in the center, facilitating uniform spheroid formation. This setup mimics in vivo conditions better than 2D cultures, making it ideal for cancer and stem cell research.

2. **What cell density should I use for spheroid formation?**

   The optimal cell density depends on the cell type and the desired spheroid size. Typically, densities range from 500 to 5,000 cells per well. A pre-experiment to test different densities will help determine the ideal conditions for your specific cell line.

3. **How do I ensure consistent spheroid size across wells?**

   - Using an automated cell counter to ensure accurate seeding.
   - Mixing the cell suspension thoroughly before seeding.
   - Ensuring uniform distribution and avoiding edge effects by filling the outer wells with sterile PBS or media.

4. **How do I monitor spheroid growth and quality?**

   Regularly observe spheroid morphology using an inverted microscope. Spheroids should be spherical, with smooth, well-defined edges. Viability assays like Live/Dead staining or ATP-based assays (e.g., CellTiter-Glo) can assess cell health.

5. **How to change media for spheroids?**

   Change media partially (usually around half) to avoid disturbance to spheroids and maintain secreted factors important for spheroid growth. Check spheroids under a microscope post-media change to ensure they remain intact.

Hi, This Is VivoMatter

VivoMatter BioTech is a leading company in biomaterials and advanced cell culture based in Hong Kong SAR, China. We are at the forefront of innovation, bridging biology and technology to drive scientific advancement. Our multidisciplinary team of biologists, chemical engineers, and material scientists is dedicated to developing state-of-the-art 3D cell culture solutions.

Specializing in advanced 3D culture technologies, VivoMatter BioTech aims to deliver high-quality materials that empower cancer and stem cell research. Our mission is to provide cutting-edge tools that enable researchers to explore new frontiers in biomedical science.

Looking for more? Please visit us at www.vivomatter.com.

You may also follow us on WeChat. (微信搜索 “博迈生物”)

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