In vivo Tumor Growth with Vivogel ™ Matrix

Application Note

Catalog #: VM001-10, VM001-PRF-10

Package size: 10 mL

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.

A cell-derived xenograft (CDX) model is the gold standard for anti-cancer therapies. The response of xenografts obtained in immune-deficient animals in drug testing is comparable to that in clinical practice, which makes xenograft models robust research tools in oncology. This note demonstrates that **Vivogel ™ Matrix** has the ability to promote cell-derived xenograft formation in nude mice.

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**

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
BALB/c Nude Mouse		
Lewis lung carcinoma	ATCC	CRL-1642

B. Prepare cells

1. Seed cells into 10 cm dishes (0.5 x 10^6 cells/dish) three days before implantation.
2. Change media (DMEM/F12 + 10% FBS + 1% Pen/Strep) the day before implantation.

Note: Cell activity is essential to the success of xenograft establishment. Keep culture media fresh.

3. Harvest cells by trypsinization, rinse with PBS three times, count and resuspend cells in PBS at 1 x 10^7/ml.

   Note: Total volume of cells prepared will depend on the number of mice needed.

4. Prepare 1.5 ml Eppendorf tube per mouse and mix 60 µl cell suspension in PBS with 60 µl Vivogel (the common ratio of cells:Vivogel is 1:1).

   Note: Since Vivogel forms a gel above 10 °C, Vivogel solution should be thawed overnight at 4 °C and kept on ice throughout the preparation. Tubes and tips for transferring Vivogel solution should also be chilled prior to implantation. Also, increasing cell density or Vivogel ratio usually benefits the experiment. You may also use Vivogel to resuspend the cell pellet for injection, but remember to keep everything cool.

C. Implant cells and Matrigel mixture into BALB/c Nude Mouse

1. Anesthetize mouse by isoflurane inhalation. Keep mouse warm and monitor breathing during procedure.

   Note: You should follow the IACUC protocol to administer the right dose of isoflurane.

2. Disinfect injection site with 70% ethanol for 3 times.

3. Inject the mixture into a mouse subcutaneously. An appropriate needle size (21-25G) should be selected to prevent the destruction of cells.

D. Monitor tumor growth

1. Check mouse health daily.

2. Measure tumor size using a caliper 3 times/week and weigh mouse until tumor size reaches around 1,000-1,500 mm^3 (Note: endpoint will depend on the goal of the experiments). Three parameters of the tumor: the length (the longest diameter), the width (the diameter perpendicular to the length), and the height were recorded.

3. Drugs could be administered at appropriate time if needed.

E. Harvest primary tumor tissues and organs

1. Perform CO_2 euthanasia and maintain CO_2 flow for a minimum of 1 min after respiration ceases; then perform cervical dislocation.

   Note: You should follow your IACUC protocol to sacrifice mouse.

2. Clean tumor area with 70% ethanol.

3. Cut skin and remove tumor carefully and place tumor in a sterile tube and keep it on ice.

4. Harvested tumor could be further stained and characterized.

Representative results:

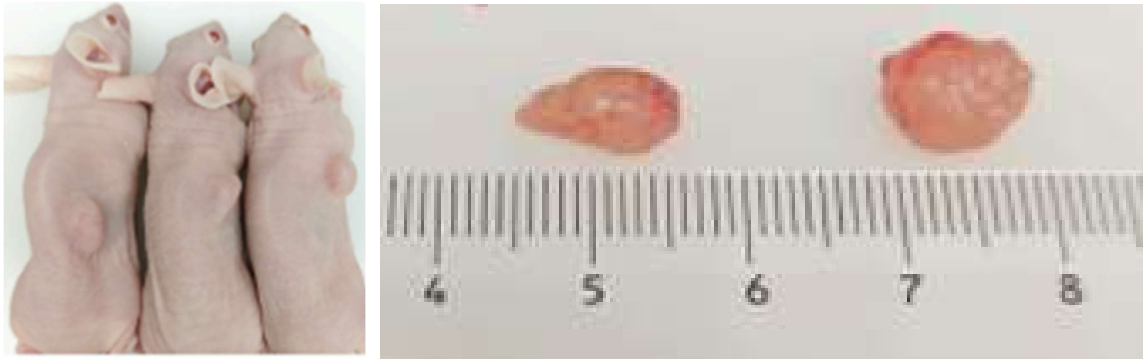

Figure 1. LLC xenograft formed in Nude mice.