Endothelial *In vitro* Angiogenesis (Tube Formation Assay) with Vivogel ™ Matrix

Application Note

Catalog #: VM001-10, VM001-PRF-10, VM002-10, VM002-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.

Angiogenesis is a vital process for normal tissue development and wound healing, but is also associated with a variety of pathological conditions. Using this protocol, angiogenesis may be measured in vitro in a fast, quantifiable manner. Several types of endothelial cells can be used for this assay including both primary cells and immortalized cell lines. The cell line used for this article was HUVEC (human), but the same methodology can be applied with other endothelial cell lines such as SVEC4-10 (mouse) or 3B-11 (mouse) cells. Depending on which cell line is used and whether the endothelial cells are transformed or non-transformed, optimization will need to be conducted to identify the ideal time needed for proper tube formation.

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, VM001-PRF-10, VM002-10, VM002-PRF-10
HUVEC cells	ATCC	CRL-1730
EGM™ 2 Growth Medium	Lonza	CC-3162

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B. Procedures

1. **Seed endothelial cells and allow them to grow.** Plate endothelial cells in an appropriate culture vessel using endothelial cell growth medium. Use seeding densities between 5×10^3 cells/cm^2 and 2×10^4 cells/cm^2 as recommended in the Lonza product manual. Replace culture medium every 2–3 days. Allow the cells to reach 70 – 90% confluency.

2. **Thaw Vivogel.** One day before 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. **Coat 96-well plate with Vivogel.** Place a tube of fully thawed Vivogel on ice. Invert the tube for a few times. Load 50 µL of Vivogel per well of the pre-cooled 96-well plate. Vivogel should be evenly distributed across each well. Incubate the 96-well plate in a humidified incubator (37 °C, 5% CO_2) for 30 min - 1 hour to allow complete gelation.

4. **Prepare endothelial cells for tube formation assay.** Endothelial Cells should be 70 – 90% confluent. Harvest endothelial cells and resuspend in culture medium containing 0.5~10% serum or your desired angiogenesis stimulators at 1~2 $\times10^5$ cells/mL. Add 150 µL (=1.5~3$\times10^4$ cells) of each single cell suspension per well on top of the gelled Vivogel. Be careful not to touch the surface of the gel.

5. **Incubate at 37 °C.** Incubate the 96-well plate in a humidified incubator (37 °C, 5% CO_2) for 4 to 18 hours. Cells can be monitored at desired time points using an inverted microscope.

Representative results:

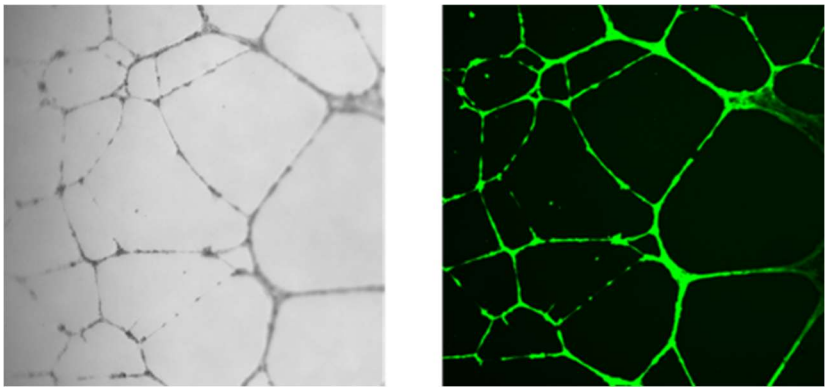

Figure 1. Angiogenesis of human umbilical cord endothelial cells (HUVECs) on Vivogel Matrix. Stained with Fluorescein Diacetate (FDA).

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