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## Methods for Implantation of BD Matrigel™ Matrix into Mice and Tissue Fixation

<sup>1</sup>Kazuo Ohashi, M.D., Ph.D., <sup>1</sup>Takashi Yokoyama, M.D., <sup>1</sup>Yoshiyuki Nakajima, M.D., Ph.D., and <sup>2</sup>Marshall Kosovsky, Ph.D.  
<sup>1</sup>Nara Medical University, Nara City, Nara JAPAN; <sup>2</sup>BD Biosciences – Discovery Labware, Billerica, MA

## Introduction

BD Matrigel™ Matrix is a solubilized basement membrane preparation extracted from Engelbreth-Holm-Swarm (EHS) mouse sarcoma, a tumor rich in ECM proteins. Its major component is laminin, followed by collagen IV, heparan sulfate proteoglycan, and entactin. BD Matrigel Matrix is effective for the attachment and differentiation of both normal and transformed anchorage-dependent epithelial and other cell types.

BD Matrigel Matrix is highly useful in various studies including 3D cell culture, cell invasion and migration assays, drug metabolism/toxicology, *in vitro* and *in vivo* angiogenesis assays. This report describes the use of BD Matrigel Matrix for *in vivo* applications such as angiogenesis and human tumor cell implantation in mice.<sup>1-8</sup>

1. BD Matrigel Matrix (*Cat. Nos. 354234 and 356234*) is suitable as a scaffold for supporting the implantation of various tumor cells. Growth Factor Reduced (GFR) BD Matrigel Matrix (*Cat. Nos. 354230 and 356230*) is also available for studies in which a reduced growth factor composition is required.
2. BD Matrigel Matrix phenol red-free (*Cat. No. 356237*) and GFR BD Matrigel Matrix, phenol red-free (*Cat. No. 356231*) are typically used for the cyan-metrohemoglobin method that measures hemoglobin content (measurement of reddish-brown absorption) in angiogenesis studies. BD Matrigel Matrix has been shown to enhance the process of angiogenesis *in vivo*.
3. BD Matrigel Matrix High Concentration (HC) (*Cat. No. 354248*) is suited for *in vivo* applications where a high protein concentration augments growth of tumors. The high protein concentration (18-22 mg/ml) also allows the BD Matrigel Matrix plug to maintain its integrity after subcutaneous injection into mice. This keeps the injected tumor cells and/or angiogenic compounds localized for *in situ* analysis and/or future excision.

## Procedures

## Subcutaneous injection of BD Matrigel Matrix into a mouse

1. Since BD Matrigel Matrix forms a gel above 10°C, BD Matrigel Matrix solution should be kept at low temperatures, and thus all equipment and reagents (syringes, needles, BD Matrigel Matrix solution, etc.) should be chilled on ice prior to injection.
2. After mixing BD Matrigel Matrix with a cell suspension [*Note 1*], the BD Matrigel mixture is injected into a mouse subcutaneously [*Note 2*] (*Figure 1*). An appropriate needle size (21-25G) should be selected to prevent the destruction of cells. To increase the contact area of the injected BD Matrigel mixture into subcutaneous tissues, a wide subcutaneous pocket should be formed by swaying the needlepoint right and left after a routine subcutaneous insertion. The BD Matrigel mixture is then injected into the pocket. When the BD Matrigel mixture is injected into a particular area without swaying the needlepoint, the mixture will form a large cell clump and a subsequent growth defect may result due to inefficient perfusion of nutrients to the cells within the core of the clump.

**Note 1:** In this experiment, undiluted BD Matrigel Matrix alone was injected into the mouse. For tumor implantation applications, approximately  $2 \times 10^7$  cells/ml of cell suspension should be mixed with BD Matrigel Matrix, resulting in a final cell concentration of  $\sim 10^6$  cells/ml. To prevent incomplete gel formation in mice, do not dilute BD Matrigel Matrix to a final concentration below 4 mg/ml.

**Note 2:** In this experiment, 0.7 ml of the BD Matrigel was injected. The injection volume of BD Matrigel takes into account the absorption of BD Matrigel into the tissue and allows for easy removal of the resultant tissue 'plug'. The optimal injection volume should be determined according to the requirements of your experiment.

While the injection of  $\sim 0.1$  ml of a BD Matrigel mixture into mice may be sufficient for the augmentation of tumor growth, the injection of at least 0.5 ml is recommended for *in vivo* angiogenesis studies.



**Figure 1.** Subcutaneous injection site.

## Removal of the BD Matrigel plug from the mouse

3. After an appropriate incubation period [*Note 3*], the mouse is anaesthetized and a square segment of tissue is excised with scissors. To ensure complete excision of the plug, cut  $\sim 5$  mm wider than the implantation site on all sides. To maintain the shape of the BD Matrigel plug, excise the subcutaneous tissue, peritoneum, as well as skin. These tissues are then fixed with formalin. *Figure 3* shows the implanted BD Matrigel viewed from the peritoneal side following excision. The volume of the implanted BD Matrigel is reduced from the injected volume due to absorption and partial degradation of BD Matrigel *in vivo*. The excised BD Matrigel plug is usually clear yellowish in color. If blood vessels are formed within the BD Matrigel plug, the color of the BD Matrigel will appear red (*Figure 4*).

**Note 3:** In this experiment, the BD Matrigel plug was removed after one week. When the quantity of hemoglobin is used to assess angiogenesis, BD Matrigel containing VEGF and heparin should be injected to promote angiogenesis. After about three days, the BD Matrigel plug containing newly formed blood vessels can be easily removed.



**Figure 2.** An arrow indicates the BD Matrigel™ injection site. A square indicates the excised region for the sample.



**Figure 3.** The implanted BD Matrigel™ plug viewed from the peritoneal side (needlepoint).



**Figure 4.** The removed BD Matrigel™ plug from the subcutaneous tissue.

### Fixation of tissues including BD Matrigel Matrix

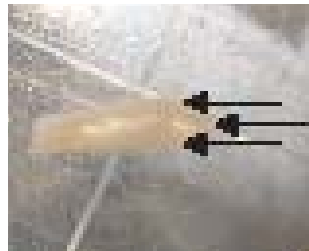
4. The excised tissue should be stretched and put on a sheet of thick paper (e.g., poster board) to avoid the formation of wrinkles. The tissue is then placed in a nylon bag for protection. Fix the tissue in 10% neutralized formalin solution for at least one day at room temperature [Note 4]. This treatment will harden the tissue in preparation for slicing the sample (Figure 6). Care should be taken to ensure that the thickness of the slice is adequate to retain the implanted BD Matrigel plug.

**Note 4:** Fixation of BD Matrigel under 8°C may cause depolymerization of BD Matrigel. Therefore, BD Matrigel should be fixed at room temperature.

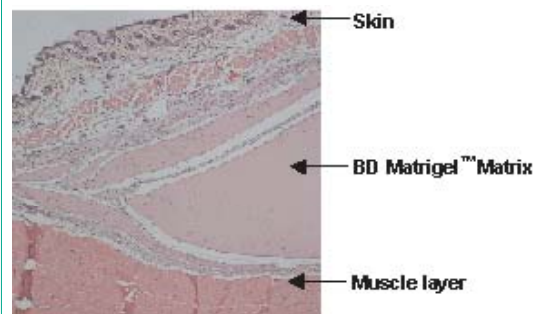
5. The fixed BD Matrigel plug can be embedded in paraffin to prepare sections for histochemical staining. Figure 7 shows a section of the BD Matrigel plug stained with hematoxylin-eosin (HE). BD Matrigel appears pink to light reddish in color with HE staining.



**Figure 5.** The removed tissue was fixed in a nylon bag. The skin side is facing upward.



**Figure 6.** Arrows indicate the cut surface of the fixed BD Matrigel™ plug. (Upper arrow = Skin; Middle arrow = BD Matrigel; Lower arrow = muscle layer).



**Figure 7.** HE stain image.

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**BD Biosciences**  
Two Oak Park  
Bedford, MA 01730 USA  
tel: 877.232.8995  
fax: 800.325.9637

**BD**  
Akasaka Garden City,  
Akasaka 4-15-1, Minato-ku,  
Tokyo, 107-0052 Japan  
tel: (81) 24 593 5405  
fax: (81) 24 593 5761

**BD Biosciences**  
Erembodegem-Dorp 86  
9320 Erembodegem, Belgium  
tel: (32) 53 720 211  
fax: (32) 53 720 450  
contact\_bdb@europe.bd.com

**BD**  
2280 Argentia Road  
Mississauga, Ontario  
Canada L5N 6H8  
tel: 866.979.9408  
fax: 800.565.0897

**BD Biosciences**  
Singapore Branch  
30 Tuas Avenue 2  
Singapore 639461  
tel: (65) 6861 0633  
fax: (65) 6860 1590

**BD Biosciences**  
4 Research Park Drive  
Macquarie University Research Park  
North Ryde NSW 2113 Australia  
tel: (800) 656 100  
fax: (612) 8875 7200  
aus\_customerservice@bd.com