



# Use of Trevigen® Cultrex® Basement Membrane Matrix to improve take and growth of xenografts in mice.

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## Introduction

One of the most challenging aspects of modeling human cancers in animals is the achievement of tumor growth of cancer cell lines, cancer stem cells or tumor biopsies implanted in animal hosts. Most frequently, mice are used as recipients of human cancer cells and biopsy specimens, and the model is collectively known as xenograft or tumorgraft. Amongst the xenograft and tumorgraft models, we find the PDXs or patient derived xenografts. PDXs involve the extraction of primary cells derived from tumors or small pieces of tumor tissue of human patients and the implantation of these cells into the recipient animal. Tumorgrafts/PDXs were developed almost fifty years ago, but even after the recent development of highly immunodeficient strains of mice that will not reject the implanted cells, positive results from grafting experiments remain uncertain. One promising advance was the finding, almost three decades ago, that the co-injection of Basement Membrane Matrix or Cultrex BME with tumor cells improved tumor take and growth, and since, a number of cell lines have shown increased take and growth when mixed and co-injected with Cultrex BME.

In the past, researchers have used our original formulation of Cultrex BME in their xenograft experiments, now Trevigen offers **Cultrex Basement Membrane Matrix BME Type 3** that has been developed, produced, and qualified specifically for use in *in vivo* studies. Cultrex BME Type 3 mimics the *in vivo* microenvironment, including increased stiffness, low glucose, and low pH, to improve take rate and growth of implanted cells for xenograft and tumorgraft models. Some of the quality control specifications of Cultrex BME Type 3 include:

- PathClear® denomination: it has tested negative by PCR for 17 bacterial and virus strains typically included in mouse antibody production (MAP) testing, plus 13 additional murine infectious agents, including LDEV, for a total of 31 organisms and viruses;
- Very low levels of endotoxins:  $\leq 8$  EU/ml measured by LAL assay;
- Provided at a protein concentration of 12 - 18 mg/ml;
- Provided in RPMI medium with 10  $\mu$ g/ml gentamycin.

Yet, the use of Cultrex BME may not entirely solve problematic or slow growing tumors, as other possible causes may include the mice strain used and the tumor or cell processing technique chosen to perform the experiment. The following tips were designed to solve frequently asked questions from researchers working with stalled or slow growing xenografts.





## Tips to improve initiation and growth of tumor cells in mice

### 1. How to process the starting material and improve reproducibility.

#### a. Tissue from tumor biopsies:

- It is extremely important that all surgically removed tissue or biopsy material is used as soon as possible after harvest, within 24 hours. Keep and transport material at 4°C in tissue culture medium without serum but containing 1-5% Cultrex BME for improved take and growth.
- Using a scalpel or razor, finely mince the biopsy to about 1 mm cubes.
- Implant 1 to 10 cubes per injection site suspended in BME using a trocar or an 18 gauge needle. Take into consideration that only tumor material will grow, implanted healthy normal cells do not proliferate. Also note that the growth takes place along the cut edges, so smaller pieces are preferred to larger ones.

#### b. Cancer stem cells and primary or established tumor cell lines:

- If a suspension of single cells is injected, make sure that the culture is exponentially growing and viable on the day of the experiment (perform a quick trypan blue test to check viability of the cells).
- Do not use cells if they appear more than 80% confluent.
- Always passage cells at least twice after thawing from liquid nitrogen before implantation.
- As a considerable amount of material is lost between injections and during loading of each needle, have twice as many cells growing as you calculate will be needed.
- Be sure cells are passaged or fed the day before the injection.
- Do not over trypsinize the cells.
- Be prompt and inject cells right after they are ready (i.e. have everything set up and ready to start once the suspension of cells is made). Cells lose viability if maintained as a suspension at low temperatures for long periods of time.
- Settling of cells occurs in the bottom of the tube, so mix the cells gently but thoroughly before loading the syringe each time.

**2. How many cells and how much volume to inject.** The optimal amount of material or number of cells injected will vary in every case, as the reported number of cells used ranges from 1 to 10 million cells. In Table 1, some examples from the literature are shown. It is also important to note that some specific cell types may be injected as a complex mix, for example, involving both tumor epithelial and healthy stromal cells. Always try to use published papers as a guide to the number of cells, location, and type of recipient mouse based on your needs. There are no set rules.

**The optimum number of cells to be injected is determined by three variables:**

- a. Type of tumor cell** (slow growing vs. metastatic, with or without additional cells such as stromal cells)
- b. Location of injection** (subcutaneous vs. orthotopic)
- c. Type of mouse** (Low immunodeficiency-Nude to high immunodeficiency-NSG™)



Table 1. Examples of xenografts in the literature.

Type of cell	Number of cells	Volume injected	Site of injection	Final concentration of BME	Mouse strain	Reference
<b>Modified human mammary epithelial Melanoma</b>	$2 \times 10^6$	200 $\mu$ l	SC	7 mg/ml	Nude	Elenbaas, 2001
<b>Human SET-2 leukemia MDA-MB-231</b>	1	100 $\mu$ l	SC flank	3 mg/ml	NOD scid	Quintana, 2008
<b>Human SET-2 leukemia MDA-MB-231</b>	$5 \times 10^6$	100 $\mu$ l	SC flank	7 mg/ml	SCID beige	Hart, 2011
<b>MDA-MB-231</b>	$0.5 \times 10^6$	100 $\mu$ l	Mammary gland	7 mg/ml	Nude	Puchalapalli, 2016
<b>PDX Colorectal cancer</b>	3 x 3 x 3 mm	25 $\mu$ l estimate	SC flank	10 mg/ml	Nude	Gock, 2016
<b>MCF7 + Fibroblasts</b>	$1.5 \times 10^6 + 10^6$	500 $\mu$ l	SC	5 mg/ml	Nude	Noel, 1994

**Choosing the right implantation site.** The site of injection chosen is also of importance, but any location that suits your research is acceptable. Subcutaneous implantations on the flank of animals are the most common choice, as they are fast and easy. The back of the neck is highly vascular and is also a good location for injection. An orthotopic site might also be considered as more physiological.

- a. **Subcutaneous xenografts.** A recommended site to inject the cells is the upper region of the back near the neck as it contains a considerable amount of fat tissue. This is a highly vascularized area, good for tumor growth, and hard to reach for mice. Usually inject 100 to 300  $\mu$ l of tumor cells or biopsy plus BME, at a final BME concentration of 7 to 10 mg/ml for optimal results.
- b. **Orthotopic site (e.g. mammary fat pad, brain, pancreas, liver, etc.).** It may result in a more relevant model, and it may increase the chances of physiological metastasis. Inject a final volume of 20 to 100  $\mu$ l of tumor cells or biopsy plus BME at a final BME concentration of 7 to 10 mg/ml for optimal results.

**3. How to use Cultrex BME and co-inject it with the cells.** If this is the first time that you have used Cultrex BME, there are some important issues to note before continuing. Cultrex BME is a soluble form of basement membrane purified from the Engelbreth-Holm-Swarm (EHS) tumor. This extract provides a natural extracellular matrix hydrogel that is liquid at 4°C and polymerizes at 24-37°C to form a reconstituted basement membrane. Basement membranes are continuous sheets of specialized extracellular matrix that form an interface between epithelial, endothelial, muscle, or neuronal cells and their adjacent stroma and play an essential role in tissue organization by influencing cell adhesion, migration, proliferation, and differentiation. The major components of Cultrex BME include laminin, collagen IV, entactin, and heparan sulfate proteoglycans. Cultrex BME is sterile, mycoplasma-free and LDEV-free. Because Cultrex BME has this particular property of staying in a liquid form at 4°C but rapidly gelling when the temperature rises, all the steps must be performed on ice, and all material should be pre-chilled and kept cold at all times. Partially gelled samples will be difficult to inject. But why do some cells grow better when co-injected Cultrex BME? Cultrex BME is prepared from a tumor and contains all of the components found in authentic basement membrane matrix. Among these components, we can find a series of growth factors and ligands that can stimulate growth and attachment of tumor cells.

#### Procedure for tumorgraft/xenograft injections using Cultrex BME:

- Thaw a vial of Cultrex BME overnight at 4°C (put the vial on ice in an ice bucket with a cover in the refrigerator), dispense in working aliquots for future use. Keep aliquots at -80°C and avoid more than 3 thaw-freeze cycles.
- The day before the injection, thaw an aliquot of Cultrex BME overnight at 4°C. Tip: if you have to thaw the Cultrex BME on the day of the experiment, roll vial between your hands trying to avoid warming up the BME too much and causing gelling. Immediately put on ice.
- Pre-chill on ice: tubes, cells, medium, syringes, needles, and all material that could be in touch with the BME to avoid polymerization.



- Pellet cells, or finely minced biopsy material, for 5 minutes in a tabletop centrifuge at room temperature. Tip: You can prepare a tube of cells or tissue for multiple injections. Finely chopped biopsy material must fit in an 18 gauge needle.
- Decant supernate, tap tip of tube with your finger to disperse pellet, and put tube on ice. Keep everything on ice for all future steps.
- Note the volume, and add an equal amount of thawed Cultrex BME on ice. Tip: Make up about 15% extra material as some is lost in the syringe and you want to be sure to have enough for each animal. Generally, use 6-8 mice per data point.
- Avoid introducing bubbles into the Cultrex BME. As Cultrex BME is a viscous extract, try to manipulate it gently and always mix it slowly by inversion. After thawing and diluting cells, mix well by inversion a few times.
- Mix well but gently to avoid cell/tissue damage and bubbles, load a pre-cooled syringe and immediately inject.
- To avoid settling of the cells in the syringe and gelling of Cultrex BME, it is recommended to load each syringe with enough cells-BME volume to inject no more than two mice. Tip: Between injections is advisable to invert the syringe up and down and to try to work as quickly as possible. Use a 25 gauge needle if possible as a smaller bore will produce less leaking. At this point, you do not need to worry about sterility. When injecting subcutaneously use the full length of the needle so that the injected material is more distant from the entry hole.
- As a general rule, use 100 to 300  $\mu$ l per injection for a final concentration of 7-10 mg/ml of BME.
- Hold the syringe in place for 10-20 seconds to allow Cultrex BME to begin to gel. Tip: Do not reuse the needle or the syringe as some gelling may have occurred. For the larger bore needle, wait longer for the gelling to occur. There will be a “bump” where the tumor cells are injected.
- Gently remove needle of the syringe with rotation to seal the hole. Some leaking may occur. Tip: If female mice are used they can be grouped in cages. Male mice will need to be caged individually.
- Monitor tumor growth with calipers, measuring 1-3 times a week.
- Once the cells begin to grow, they degrade the BME, and the initial “bump” of BME that appears right after the injection is replaced by the actual tumor.
- Tumor growth may be observable as early as 10 days or could take up to 1 month.

**4. Choosing the recipient mouse strain.** If you are working with a cancer cell line that was never implanted in an animal before, it will be difficult to know *a priori* which mouse strain will prove to be the best.

- A preliminary study including a few animals of different immunodeficient strains is always recommended.
- It is recommended to work with female mice aged 5-6 weeks. If grouped in cages, male mice fight and injure each other. Female mice are easier to handle for the injection.
- Do not use older mice as tumors grow more slowly.
- If working with male tumors such as prostate, male mice must be used. Likewise, female cancers such as breast cancer require female mice. Some tumor cells such as MCF-7 require hormone supplementation.

**5. Bonus Tip: Boost growth with more Cultrex BME.** About 15% of biopsy material and some slow growing tumors injected with Cultrex BME will “stall” or cease growth. If stalling of the xenograft is detected, one possible option is to bypass it by re-injecting Cultrex BME to boost growth. The tumors can be rescued with a 50 to 100  $\mu$ l injection of undiluted Cultrex BME into the edge of the tumor. This approach will successfully re-initiate tumor growth, but consider that it may need to be repeated if a second stall occurs.

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