Linear Tissue Train® Culture Plates

Product Information Sheet
02/23/16 Rev. 1.1

Tissue Train® culture plates are 35 mm 6-well plates with 1) flexible silicone elastomer well bottoms and 2) anchor tabs, which are adherent to the silicone membrane, for creating three dimensional cell-seeded gel constructs (Fig. 1). The anchors come untreated or with a covalently bonded protein to improve cell attachment (Table 1). Tissue Train® culture plates can be used with the Flexcell® Tension System to create linear cell-seeded hydrogels and to apply up to 20% uniaxial tensile strain to these gel constructs. For more information, see the Tissue Train® culture plate product webpage at http://www.flexcellint.com/TissueTrainPlate.htm.

Figure 1. *Linear Tissue Train® culture plates have six 35 mm wells with either foam or nylon anchors attached to the membrane in each well.*

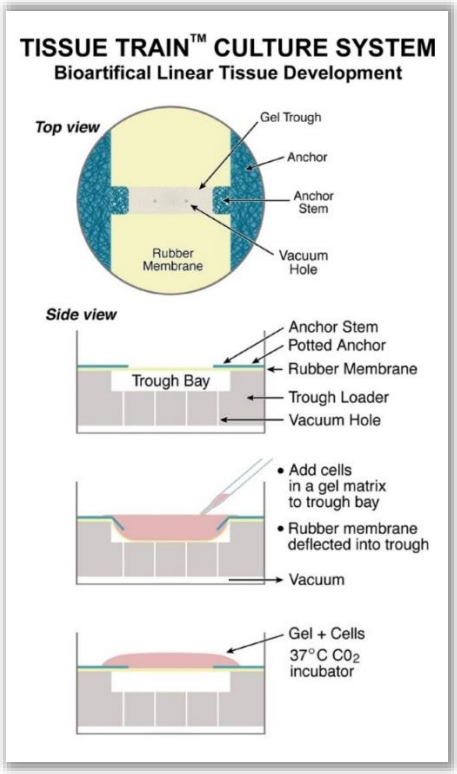

Figure 2. *Schematic of the top and side views of a Tissue Train® well with attached anchors that align along the central trough of a Trough Loader™. The vacuum holes allow the applied vacuum to deform the silicone membrane downward into the trough, thus creating a linear mold for a cell-seeded gel construct.*

PREPARATION OF CELLS IN 3D LINEAR GELS IN A TISSUE TRAIN® CULTURE PLATE

1. Prepare cells according to your established protocol for primary cultures or continuous cell lines in the medium of choice.
2. Release cells from their substrates with 0.05% trypsin, trypsin-EDTA, 0.05% bacterial collagenase, or other means.
3. Add serum containing media to the cells to neutralize the trypsin or collagenase.
4. Count cells and determine the number of cells needed, approximately 50,000-200,000 cells in 150-200 µl Collagel® or Thermacol® collagen gel for each well of a 6-well Tissue Train® culture plate. *NOTE: Cell seeding density in a 3D gel will vary depending on cell type. We recommend testing cell seeding densities to determine the best cell number for your application and cell type.*
5. Wash cells 2 times with medium to remove trypsin or collagenase.
6. Cells may be reconstituted in one volume of a hydrogel. The objective is to achieve an overall gel-MEM concentration of 1X. Before adding cells, the matrix protein gel solution should be neutralized to pH 7.0 using 1 M sodium hydroxide. The suggested formula for the Collagel® or Thermacol® 3D hydrogel is as follows: 70% by volume Collagel® or Thermacol®; 20% by volume of 5X MEM to yield an overall 1X concentration by total volume; 10% fetal calf serum; and cells.
7. Place a linear Trough Loader™ in a baseplate well. Apply a thin layer of lubricant to the top surface of the Trough Loader™. The lubricant will facilitate uniform and unrestricted conformation of the membrane into the trough.
8. Place the Tissue Train® culture plate in a red, rubber gasket atop the Trough Loaders™. Ensure that the anchor stems are aligned with the long axis of the Trough Loader™ (Fig. 2).
9. Connect to the Flexcell® FX5K Tension FlexLink® or other regulated vacuum source. Vacuum should be applied to the baseplate in a steady "hold" mode so that the flexible membrane is deformed and held in the space in the Trough Loader™. To supply the proper vacuum level with the FX-5000™ Tension System, it is recommended that a maximum of 20% elongation be used with the ***Tissue Train Plate (24mm Arctangle LS)*** platform setting. This is

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the equivalent of -90 kPa. Be sure that you allow enough vacuum tubing for your baseplate to reach from your incubator to your tissue culture hood.

10. Pipette the cell and matrix protein gel solution into the "trough" in each Tissue Train® well (Fig. 2). First pipette a small drop of gel at each end of the trough, under the anchor stems. Then press the anchor stems into the trough and release several times, thoroughly wetting the tabs. Finally, fill the middle of the trough with gel, moving the pipette back and forth to create a uniform strip of gel in the well (see video of *Tissue Train® Bioartificial Tissue Fabrication with Uniaxial Strain* on Flexcell®'s web site, www.flexcellint.com).
11. Place the baseplate with culture plates in a 37 °C incubator and allow the solution to polymerize, approximately 2 hours.
12. After the gel has set, slowly release the vacuum and add 3 ml of serum-containing media to each well. The gels should appear as a band of gel attached at each anchor end in the Tissue Train® well. Remove culture plates from the Tissue Train® baseplate, if needed.
13. Culture constructs according to the laboratories established protocol.

ORDERING INFORMATION

Linear Tissue Train® culture plates are sold individually or by the case of 40 with either Cerex (Cat. No. TT-4001) or foam (Cat. No. TT-5001) anchor tabs. Each plate is sterile and individually packaged in a sealed bag. See Table 1 for catalog numbers and corresponding protein coatings. Flexcell® culture plates have a shelf life of 1 year when stored at room temperature or 4 °C in the dark or out of direct light.

Flexcell® culture plates and Tissue Train® products are protected by the following patents: US Patents 4,789,601 and 4,822,741 (International Patents DE3855631D1, DE3855631T2, EP0365536B1); US Patent 6,048,723; US Patent 6,218,178; US Patent 6,472,202; US Patent 6,998,265.

Table 1. *Tissue Train® culture plate catalog numbers and corresponding protein coating.*

Catalog Number#	Coating*
TT-4001U/TT-5001U	Untreated
TT-4001A/TT-5001A	Amino
TT-4001C/TT-5001C	Collagen I
TT-4001C(IV)/TT-5001C(IV)	Collagen IV
TT-4001E/TT-5001E	Elastin
TT-4001L/TT-5001L	Laminin (YIGSR)
TT-4001P/TT-5001P	Pronectin (RGD)

#Tissue Train® plates with Cerex anchors have catalog numbers ending in 4001; Tissue Train® plates with foam anchors have catalog numbers ending in 5001. For anchor options, see Tech Report 113: Tissue Train® Anchor Options. Comparison between the non-woven nylon and the urethane polyester foam anchor*:* http://www.flexcellint.com/documents/113_TissueTrainAnchorTech.pdf.

**For more information on these coatings, see* Tech Report 106: Matrix Bonded Growth Surfaces. Growing Cells in a More Natural Matrix Environment: *http://www.flexcellint.com/documents/106_MatrixBondedSurfacesTech.pdf.*

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