



Trough Loaders™

Product Information Sheet

01/21/16 Rev. 1.0

Trough Loaders™ are specially designed Loading Stations™ that act as a mold when creating 3D hydrogels (Fig. 1). They are available in linear and trapezoidal shapes. For more information, see the Trough Loader™ product webpage at <http://www.flexcellint.com/TroughLoader.htm>.

CREATING 3D CELL-SEEDED GELS

1. Prepare cells in a hydrogel according to the laboratory's established protocol or with Collagel® and Thermanal® as described in their respective product information sheets available on our website, www.flexcellint.com.
2. For linear-shaped and trapezoidal-shaped constructs, use a linear or a trapezoidal Trough Loader™ and Tissue Train® culture plate, respectively. Place the Trough Loaders™ in each of the four wells of a BioFlex® baseplate.
3. 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.
4. Place the Tissue Train® culture plate in a rubber gasket atop the Trough Loaders™. Ensure that the anchor stems are aligned with the long axis of the Trough Loader™ (Fig. 2).
5. Connect to the Flexcell® Tension System 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 Arcangle LS)* platform setting. This is 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.
6. 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: <http://www.flexcellint.com/videos.htm>).
7. Place the baseplate with culture plates in a 37 °C incubator and allow the solution to gel.
8. 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.
9. Culture constructs according to the laboratories established protocol.

ORDERING INFORMATION

6-place linear Trough Loaders™ (Cat. No. TT-4000TL) and trapezoidal Trough Loaders™ (Cat. No. TTTP-4000) can be purchased in a set of 4.



Figure 1. A) Arcangle® loading post. B) Well of a UniFlex® culture plate. C) UniFlex® culture plate atop an Arcangle® Loading Station™.

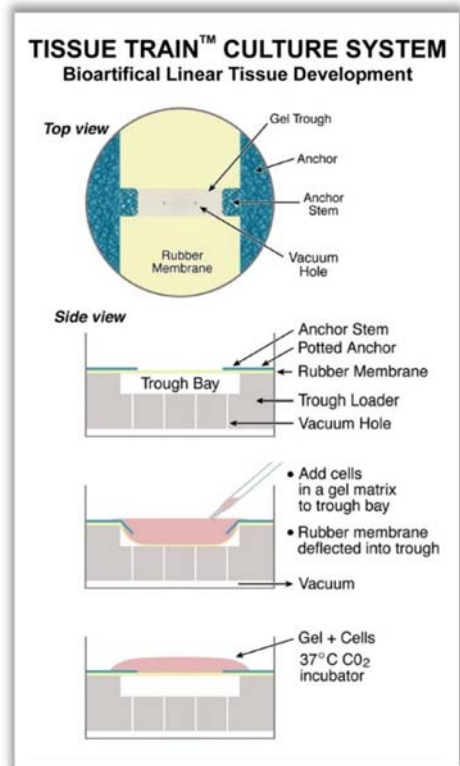


Figure 2. Schematic of the top and side views of a Tissue Train® well with attached anchors that align along the central trough of a Trough Loader™. The vacuum holes allow the applied vacuum to deform the silicone membrane downward into the trough, thus creating a mold for a cell-seeded gel construct.

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.