T-Blocks™

Product information

Tissue (T) Blocks

Tissue Blocks (T-Blocks) are porous hydrogel scaffolds with polarized microstructures that enable primary cells or immortalized cells to attach, migrate, and proliferate after seeding. As cells require more surface area to grow, additional T-Blocks may be joined to the original T-Block seeded with cells. Cells will migrate and expand within additionally joined T-Blocks. T-Blocks can be used to maintain primary cell cultures or immortalized cell cultures without the need to sub-culture when cells become confluent. See **Table 1** for instructions on how to properly store T-Blocks.

Table 1. Contents and storage

Material	Amount	Storage	Stability
PEGDA	1 vial containing 1 T-Block (1 g)	• 9 – 30 °C, Max 5-day storage • 4 - 8 °C, long-term storage • Sterile Distilled H_2O	When stored as directed, product is stable for 6 months
* Note that vial contents are hardly visible.			

Properties of T-Blocks

T-Blocks are porous hydrogel scaffolds that enable efficient growth of primary cells or immortalized cells in 3D. T-Blocks are made out of biocompatible hydrogel compositions which include proprietary blends of polyethylene glycol diacrylate (PEGDA). T-Blocks coated with extracellular matrix coatings facilitate attachment and migration of primary cell or immortalized cell populations. T-Blocks are a platform by which the T-Block substrate can be expanded by joining additional T-Blocks to the original T-Block. This process facilitates migration and proliferation of primary cells or immortalized cells, while maintaining the primary cell or immortalized cell phenotype and eliminating the requirement of sub-culture.

Materials required, but not provided.

- Sterile Saline Solution of choice
  - Hanks Balanced Salt Solution (HBSS) [without Ca^{2+} and without Mg^{2+}]
  - Phosphate Buffered Saline (PBS) [without Ca^{2+} and without Mg^{2+}]
- Extracellular Matrix coating of choice
- Primary cell or immortalized cell population of choice
- Sterile Expansion media of choice
- Sterile spatula
- Single channel pipettors
- Pipettor tips
- 50-mL Conical Tubes

Methods

Perform all procedures using aseptic technique unless otherwise noted.

- **Use the following recommendations as guidelines to determine the optimal conditions for your cell culture system.**

Pre-Soak T-Blocks

- Submerge T-Blocks in saline solution such as Hanks Balanced Salt Solution (HBSS) [without Ca^{2+} and without Mg^{2+}] or Phosphate Buffered Saline (PBS) [without Ca^{2+} and without Mg^{2+}] to activate T-Blocks one day prior to coating.

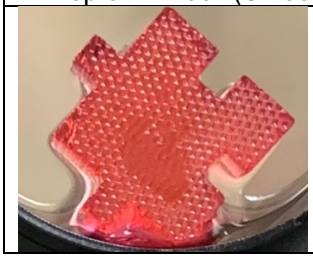
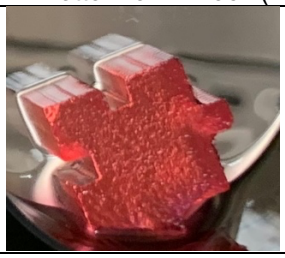
Example Protocol of Coating T-Blocks with Fibronectin

- Equalize Fibronectin to room temperature before use.
- Reconstitute fibronectin solution to a stock concentration of 1 mg/mL in HBSS [without Ca^{2+} and without Mg^{2+}].
  - Gently swirl the solution for 1 minute to mix.
    - **DO NOT VORTEX**
- Create a 50 µg/mL working fibronectin solution in HBSS [without Ca^{2+} and without Mg^{2+}].
  - Gently swirl the solution for 1 minute.
- Place T-Block in working fibronectin solution for at least 6 hours.
  - Make sure the T-Block is completely submerged.
  - T-Blocks may be incubated with fibronectin overnight.
  - T-Blocks may be coated in polypropylene conical tubes instead of well plates or mono plates.
- Remove T-Block from working fibronectin solution with sterile spatula.
- Gently wash the T-Block with sterile HBSS [without Ca^{2+} and without Mg^{2+}] three times, before seeding cells.

Seeding T-Blocks with Primary Cells

- Place coated T-Blocks in sterile non-tissue culture treated 6-well plate with the T-Block bottom “rough” side facing plate floor.
- Suspend cells of choice in media of choice at a concentration of 100,000 cells to 1,000,000 cells per 0.5 mL, dependent on cell growth rate.
- Gently pipette cell solution onto TOP side of T-Block dropwise.
  - Top of T-Block has a smooth surface (See **Table 2A**).
  - Bottom of T-Block has a “rough” appearance. (See **Table 2B**).

Table 2. Top and Bottom of T-Block after Submerged in Media

A. Top of T-Block (Smooth Appearance)	**B.** Bottom of T-Block (Rough Appearance)
	

- Wait 2 minutes, then collect the excess cell solution and gently pipette cell solution onto the TOP side of the T-Block as stated previously.
- Wait 2 minutes and repeat a third time.
  - Additional seeding cycles may be needed depending on cell type and coating
- Wait 5 minutes.
- Gradually add 6.5 - 8.5 mL of media of choice to well containing T-Block.
  - Add media against wall of well. DO NOT ADD media to top of T-Block after cells have been added.
  - Ensure that T-Block is fully submerged.
- Check T-Blocks for confluency using an inverted microscope and change media every 2 days.
  - When changing media take care not to disturb the T-Block. For best results, remove media manually with a pipette.
  - If using an aspirating system that utilizes a vacuum pump, be sure to aspirate media as far away from the T-Block as possible. DO NOT let T-Block touch aspirating tip or T-Block may be damaged.
- When cells occupy approximately 60% of all cell surfaces within T-Block, join an additional T-Block to the original T-Block by sliding the tongue of new T-Block into a groove of original T-Block as shown in **Figure 1**.
  - If needed, carefully transfer T-Block to mono-plate before adding new T-Block to provide more cell culture volume.
  - ALWAYS ensure at least one side of each T-Block is exposed to media when joining T-Blocks together to permit proper media diffusion.

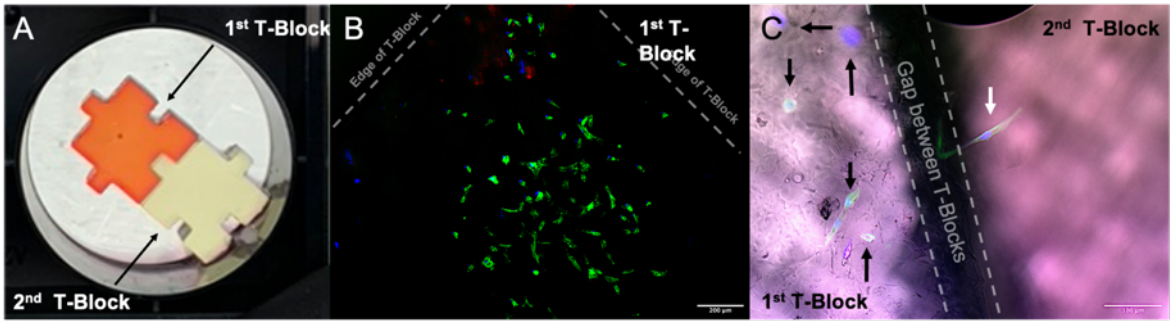


Figure 1. T-Blocks Joined Together for Cell Culture.
A) Two T-Block microstructures joined together. The 1st T-Block was seeded with 100,000 MSCs, and cultured for five days. B) MSCs detected inside 1st T-Block after seven days of culture. C) Migration of MSC between 1st and 2nd T-Block is indicated by the white arrow. Black arrows identify other MSCs. Green = Phalloidin. Red = MitoTracker. Blue = DAPI. Scale bar = 200 μm.

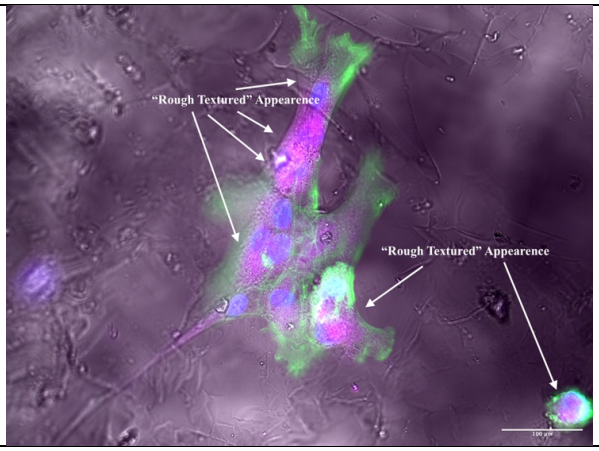
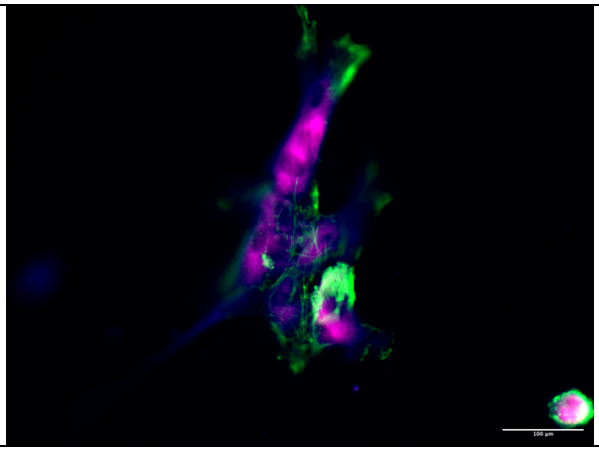
- Continue culturing T-Blocks with preferred media.
  - Ensure that enough media is added to fully submerge T-Block(s).
- Repeat process until desired cell concentration is reached.
- If dissociation of cells from T-Block is desired, use Ronawk's X-Tract Cell Retrieval Agent for best results.
- Dispose of used T-Block following standard biological hazardous wastes protocols mandated by institution, locality, state, and federal guidelines.

Supplemental Information

Cell Growth and Attachment

When T-Blocks are properly coated with extracellular matrix and seeded properly, cells will infiltrate pores and migrate throughout the T-Block. Use 10X objectives or greater when viewing cells with bright-field or epi-fluorescent microscopes to minimize light reflection within the T-Block. Cells can be identified by their "rough" texture. Use of fluorescent dyes such as Hoechst 33342 will enhance visualization of cells within T-Block dramatically. **Table 3** illustrates both transmission light with fluorescent overlay and fluorescence markers (Green = Phalloidin, Red = MitoTracker, Blue = DAPI) without transmission light. When viewing cells, keep in mind that all boundaries of cell may not be completely in focus if cell is residing in multiple Z-planes. Adjustment of the fine focus knob on the microscope may help in viewing entire cell body within T-Block.

Table 3. Visualization of T-Blocks with Cells

Transmission + Fluorescence	Fluorescence
	

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