E-Blocks™

Product information

Easy (E) Blocks

Easy Blocks (E-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 E-Blocks may be joined to the original E-Block seeded with cells. Cells will migrate and expand within additionally joined E-Blocks. E-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 E-Blocks.

Table 1. Contents and storage

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

Properties of E-Blocks

E-Blocks are made out of biocompatible hydrogel compositions which include individual and combinatorial proprietary blends of polyethylene glycol diacrylate (PEGDA) and Porcine Collagen. Coating of the E-Block is not necessary; however, an extracellular coating may be applied to the E-Block to facilitate attachment and migration of specific primary cell populations if desired. E-Blocks come in a standard configuration and are not customizable.

Materials required, provided within kit.

- Sterile spatula

Addition 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+}]
- Primary cell or immortalized cell population of choice
- Sterile Expansion media of choice
- 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 E-Blocks

- Submerge E-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 E-Blocks 30 min prior to seeding.

Seeding E-Blocks with Primary Cells

- Place E-Blocks in sterile non-tissue culture treated 6-well plate with the E-Block bottom "rough" side facing plate floor.
  - Bottom of E-Block has a "rough" appearance (See **Figure 1B**)
  - Note: To aid in determining the bottom side of the E-Block, place E-Block in plate without liquid and allow to sit for 2-5 minutes. This will allow remaining liquid to drain and remove the shimmer created by liquid-hydrogel interaction.
- *Suspend cells of choice in media of choice at a concentration of 100,000 cells to 1,000,000 cells per mL*, *seed 0.5 mL per block,* dependent on cell growth rate.
- Gently pipette cell solution onto TOP side of E-Block dropwise.
  - Top of E-Block has a smooth surface (See **Figure 1A**).

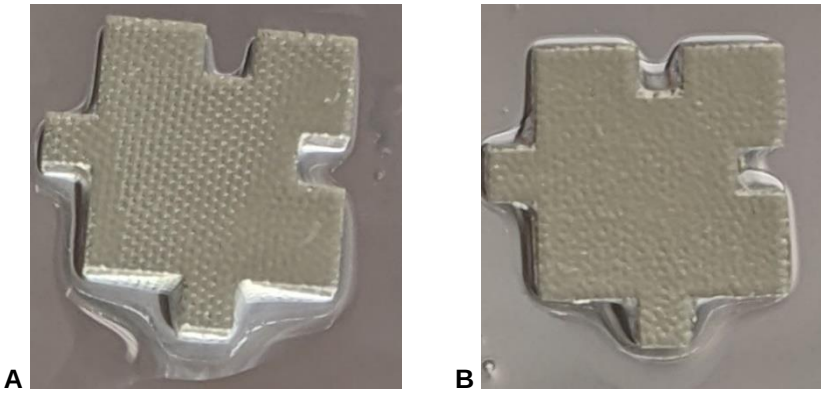


Figure 1. Top and Bottom of E-Block.
A. Top of E-Block (Smooth Appearance)
B. Bottom of E-Block (Rough Appearance)

- Wait 2 minutes, then collect the excess cell solution and gently pipette cell solution onto the TOP side of the E-Block as stated previously.
- Wait 2 minutes and repeat a third time.
  - Additional seeding cycles may be needed depending on cell type.
- Wait 5 minutes.
- Gradually add 8 mL of media of choice to well containing E-Block. Ensure that E-Block is fully submerged.
  - Add media against wall of well in a slow, controlled manner.

  - DO NOT ADD media to top of E-Block after cells have been added.
- Check E-Blocks for confluency using an inverted microscope and change media every 2 days.
  - When changing media take care not to disturb the E-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 E-Block as possible. DO NOT let E-Block touch aspirating tip or E-Block may be damaged.
  - Adding a 200-µL pipette tip to the aspirating pipette is recommended to decrease the chance of damaging the E-Block when removing media.
- When cells occupy approximately 60% of all cell surfaces within E-Block, join an additional E-Block to the original E-Block by sliding the tongue of new E-Block into a groove of original E-Block as shown in **Figure 2**.
  - If needed, carefully transfer E-Block to mono-plate before adding new E-Block to provide more cell culture volume.
  - ALWAYS ensure at least one side of each E-Block is exposed to media when joining E-Blocks together to permit proper media diffusion.

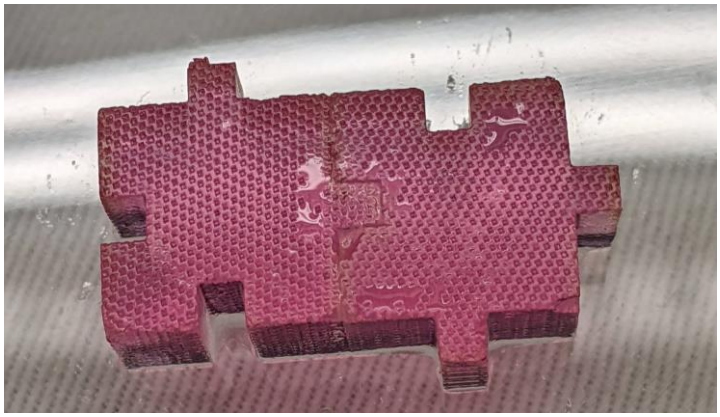


Figure 2. E-Blocks Joined Together for Cell Culture.

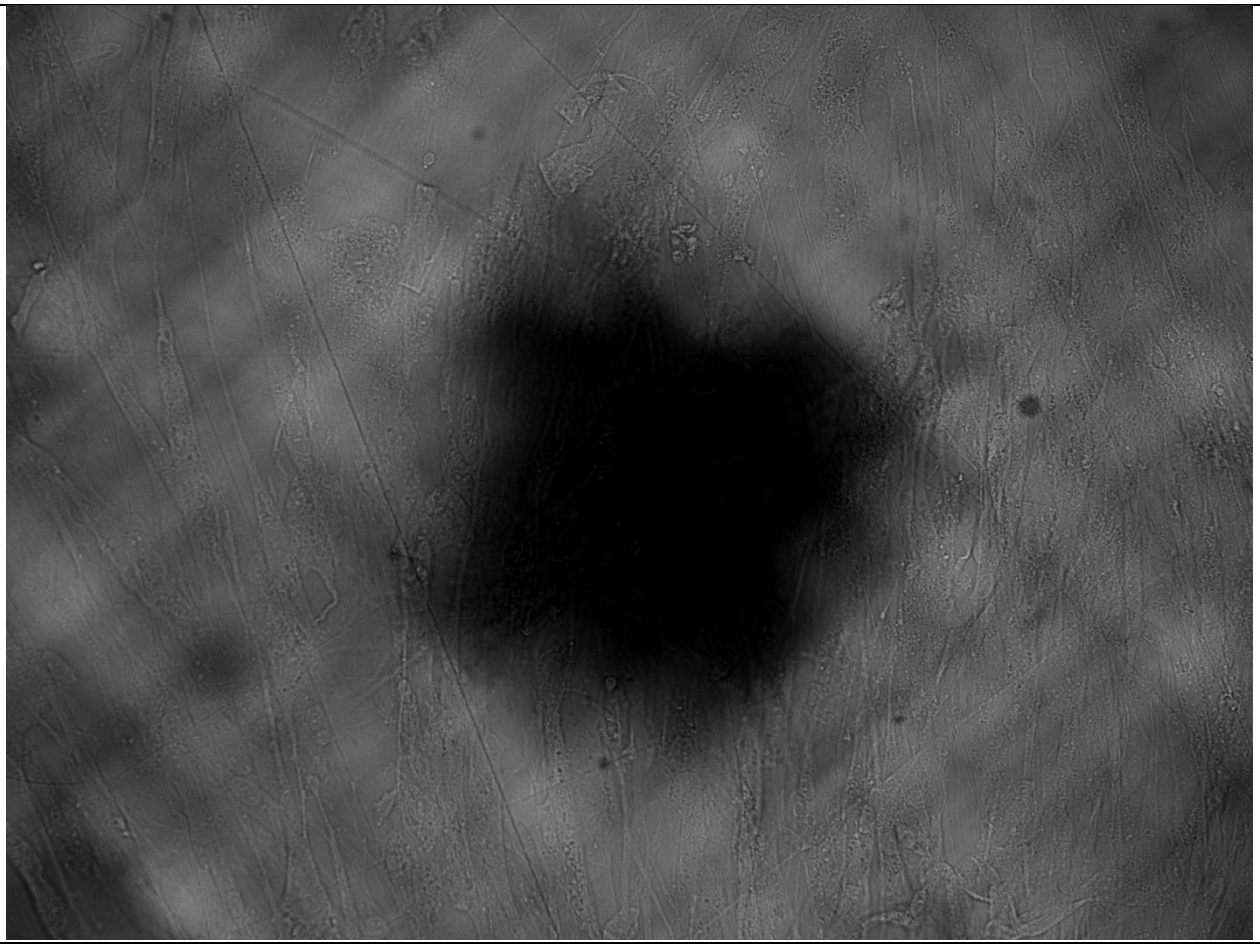
- Continue culturing E-Blocks with preferred media.
  - Ensure that enough media is added to fully submerge E-Block(s).
- Repeat process until desired cell concentration is reached.
- If dissociation of cells from E-Block is desired, use Ronawk's X-Tract Cell Retrieval Agent for best results.

Supplemental Information

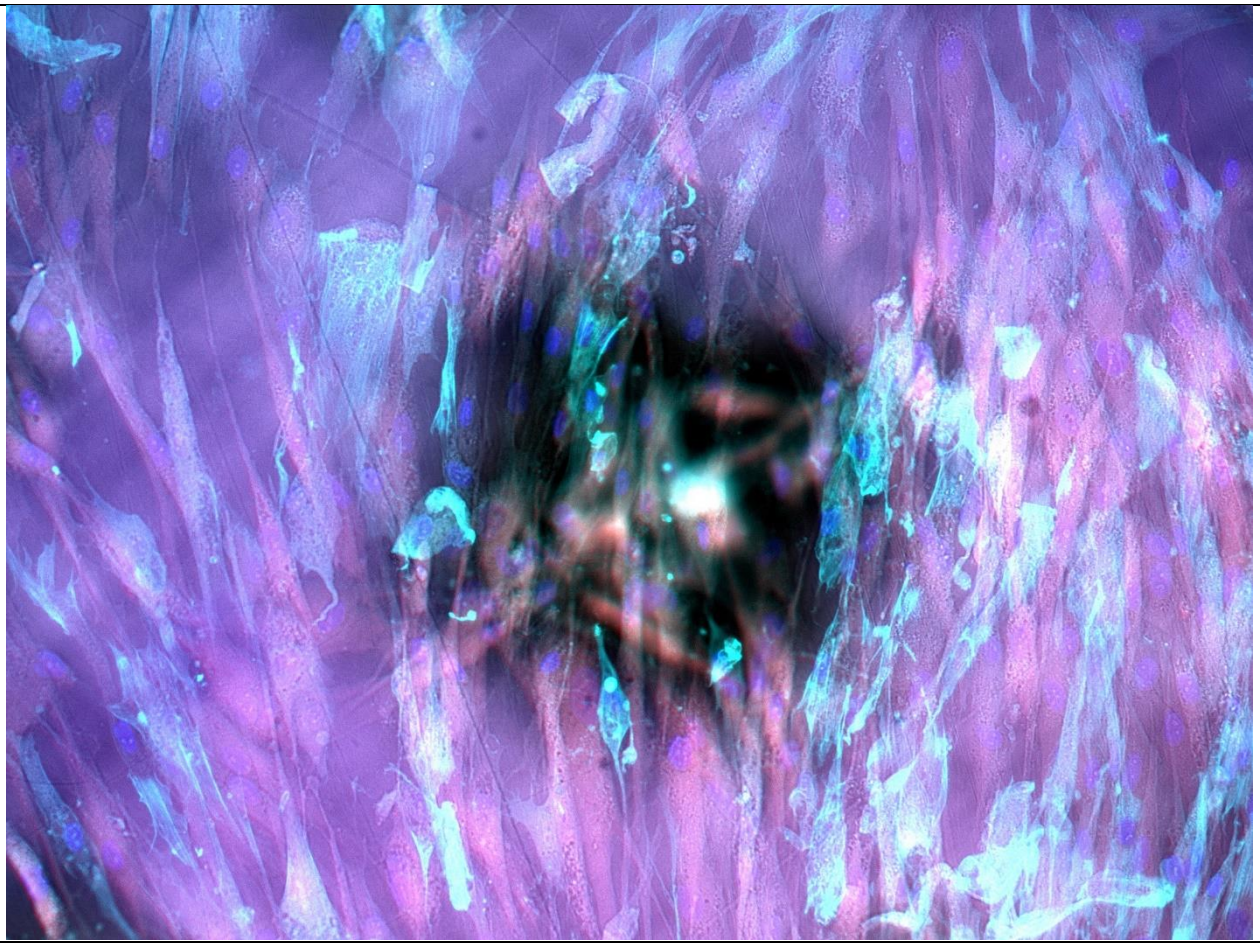
Cell Growth and Attachment

When E-Blocks are seeded properly, cells will infiltrate pores and migrate throughout the E-Block. Use 10X objectives or greater when viewing cells with bright-field or epi-fluorescent microscopes to minimize light reflection within the E-Block. Cells can be identified by their “rough” texture. Use of fluorescent dyes such as Hoechst 33342 will enhance visualization of cells within E-Block dramatically. **Table 3** illustrates cells under transmitted light (**A**), transmitted light and fluorescence markers (Green = Phalloidin, Red = MitoTracker, Blue = DAPI) (**B**) and fluorescence markers without transmission light (**C**). 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 E-Block.

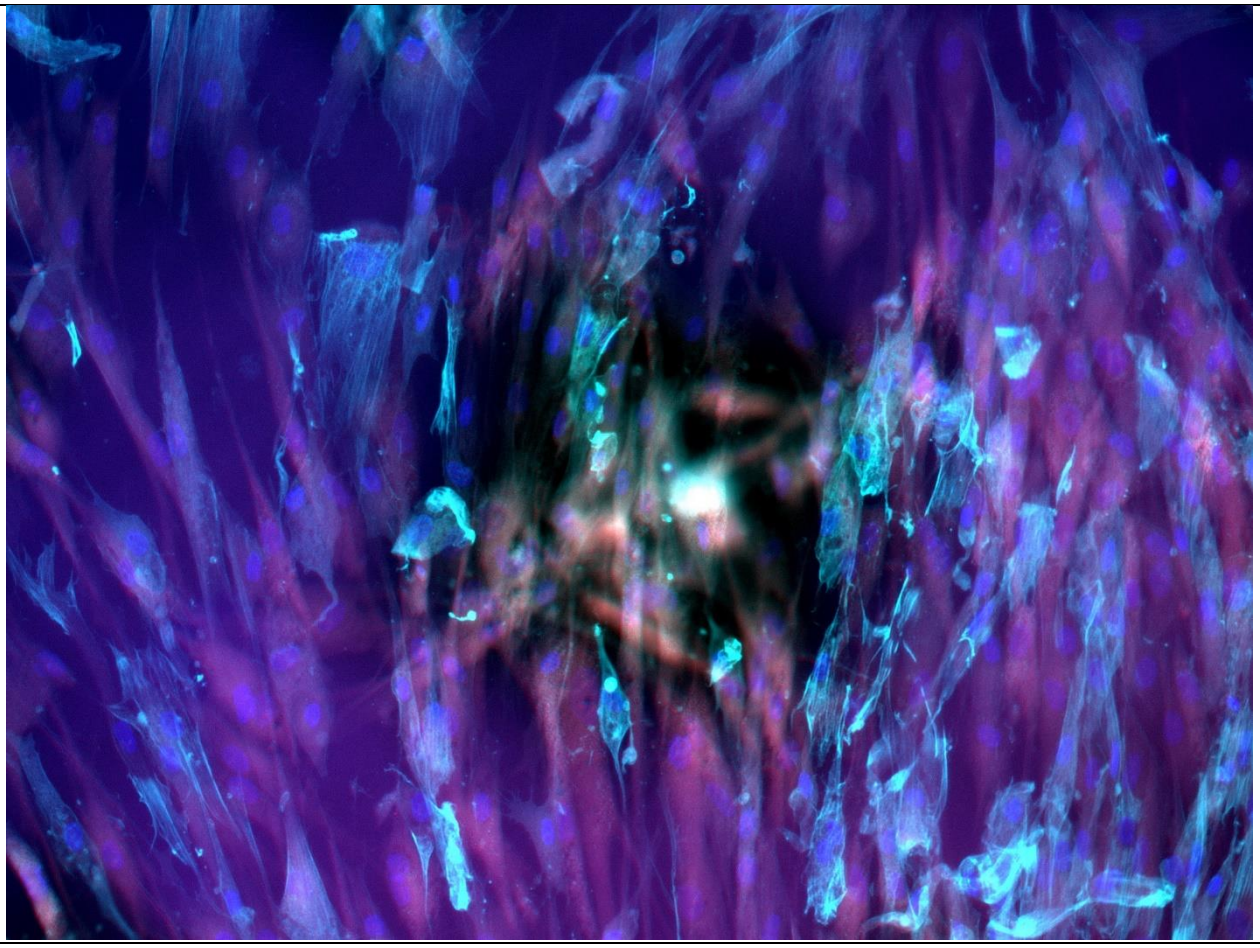
Table 3. Visualization of E-Blocks with Cells

A.) Transmission


B.) Transmission + Fluorescence



C.) Fluorescence



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