X-Blocks™

Product information

Degradable (X) Blocks

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

Table 1. Contents and storage

Material	Amount	Storage	Stability
Proprietary Blend	1 vial containing 1 X-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 3 month
* Note that vial contents are hardly visible.			

Properties of X-Blocks

X-Blocks are porous hydrogel scaffolds that enable efficient growth of primary cells or immortalized cells in 3D. X-Blocks are made from a blend biocompatible hydrogel materials. X-Blocks enable attachment and migration of primary cell or immortalized cell populations that bind to collagen. X-Blocks are a platform by which the X-Block substrate can be expanded by joining additional X-Blocks to the original X-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, provided within kit.

- Sterile spatula
- X-Tract™ Cell Retrieval Agent, in 40mg vials, store at 4-8°C

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
- Culture vessel of choice, use of non-tissue culture plate is recommended but not required
- 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 X-Blocks

- Submerge X-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+}] at room temperature to activate X-Blocks 30 min prior to seeding.

Seeding X-Blocks with Primary Cells

- Place X-Blocks in sterile non-tissue culture treated 6-well plate with the X-Block bottom "rough" side facing plate floor.
  - Bottom of X-Block has a "rough" appearance (See **Figure 1B**)
  - Note: To aid in determining the bottom side of the X-Block, place X-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 500,000 cells to 2,000,000 cells per mL*, *seed 0.25 mL per block,* dependent on cell growth rate.
- Gently pipette cell solution onto TOP side of X-Block dropwise.
  - Top of X-Block has a smooth surface (See **Figure 1A**).

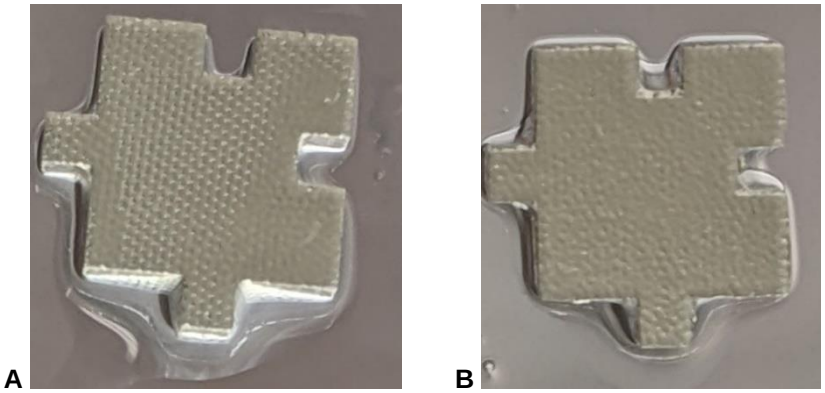


Figure 1. Top and Bottom of X-Block.
A. Top of X-Block (Smooth Appearance)
B. Bottom of X-Block (Rough Appearance)

- Wait 2 minutes, then collect the excess cell solution and gently pipette cell solution onto the TOP side of the X-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 X-Block. Ensure that X-Block is fully submerged.
  - Add media against wall of well in a slow, controlled manner.

  - DO NOT ADD media to top of X-Block after cells have been added.
- Check X-Blocks for confluency using a microscope and change culture medium every 2-3 days or as recommended for each cell type.
  - When changing media take care not to disturb the X-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 X-Block as possible. DO NOT let X-Block touch aspirating tip or X-Block may be damaged.
  - Adding a 200-µL pipette tip to the aspirating pipette is recommended to decrease the chance of damaging the X-Block when removing media.
- When cells occupy approximately 60% of all cell surfaces within X-Block, join an additional X-Block to the original X-Block by sliding the tongue of new X-Block into a groove of original X-Block as shown in **Figure 2**.
  - If needed, carefully transfer X-Block to mono-plate before adding new X-Block to provide more cell culture volume.

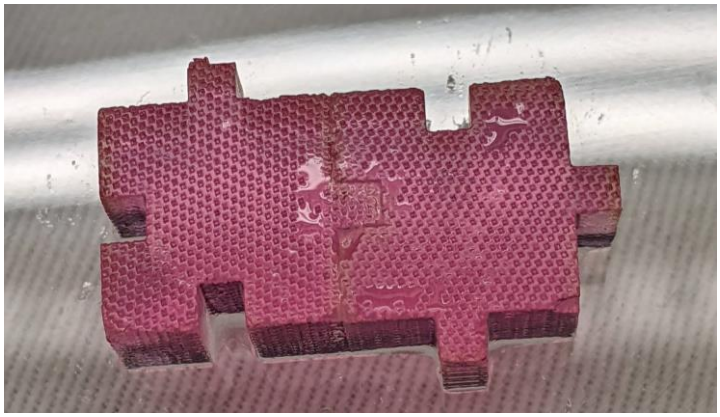


Figure 2. X-Blocks Joined Together for Cell Culture.

- Continue culturing X-Blocks with preferred media.
  - Ensure that enough media is added to fully submerge X-Block(s).
- Repeat process until desired cell concentration is reached.

<u>Dissociate cells from X-Block:</u>

- Reconstitute X-Tract Cell Retrieval Agent (see X-tract Cell Retrieval Agent Protocol for reconstitution instructions)
  - The X-tract Cell Retrieval Agent is an enzymatic reaction and recommended times may differ slightly depending on media and cell type.
- Remove culture medium and then pipette 8-10mL of pre-warmed X-Tract Cell Retrieval Solution directly on top of X-Block, ensure X-Block is fully submerged.
- Place X-Blocks in incubator for approximately 2 hours.
  - Agitation can used but is not a requirement.
  - Depending on cell and media type, it will take approximately 2 hours for the X-Block to completely dissolve.
- Remove plate from incubator as soon as the X-Block is dissolved.

- Centrifuge cells to form a pellet (adjust RCF and time per cell type accordingly).
- Remove supernatant.
- Resuspend cells in media or solution of choice.
- Count cells using method of choice.
- Plate cells or freeze immediately.

Tips on Dissociating cells from X-Block:

- The X-Tract Cell Retrieval Agent is an enzymatic reaction, so the solution must be at 37ºC to work properly.
- While it takes about 2 hours to completely dissolve the X-Block, when using the X-tract reagent for the first time, it is recommended to periodically monitor the progress of dissolution of the X-Block (approximately every 30 minutes).
    - The X-Block will gradually shrink over the course of the 2 hours. Appearance wise, the edges of the X-Block will start to round. Holes may appear in the center of the X-Blocks, which is completely normal.
- Agitation will help the enzymatic reaction but is not required. Certain cell lines do not tolerate agitation well, so proceed accordingly.
    - If agitation is not available, manual pipetting the X-Tract solution through the block every 30 minutes is recommended, but again not required.
- If you have cell clumps upon resuspension, either gently agitate or apply Accutase for approximately 10 minutes at room temperature.

Supplemental Information

Cell Growth and Attachment

When X-Blocks are seeded properly, cells will infiltrate pores and migrate throughout the X-Block. Due to the material properties of the X-Blocks, the blocks will appear fairly clear under a microscope. Cells can appear either dark or light depending on the diffraction of the light within the block. 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 X-Block.

Figure 3. X-Block - Live Cells

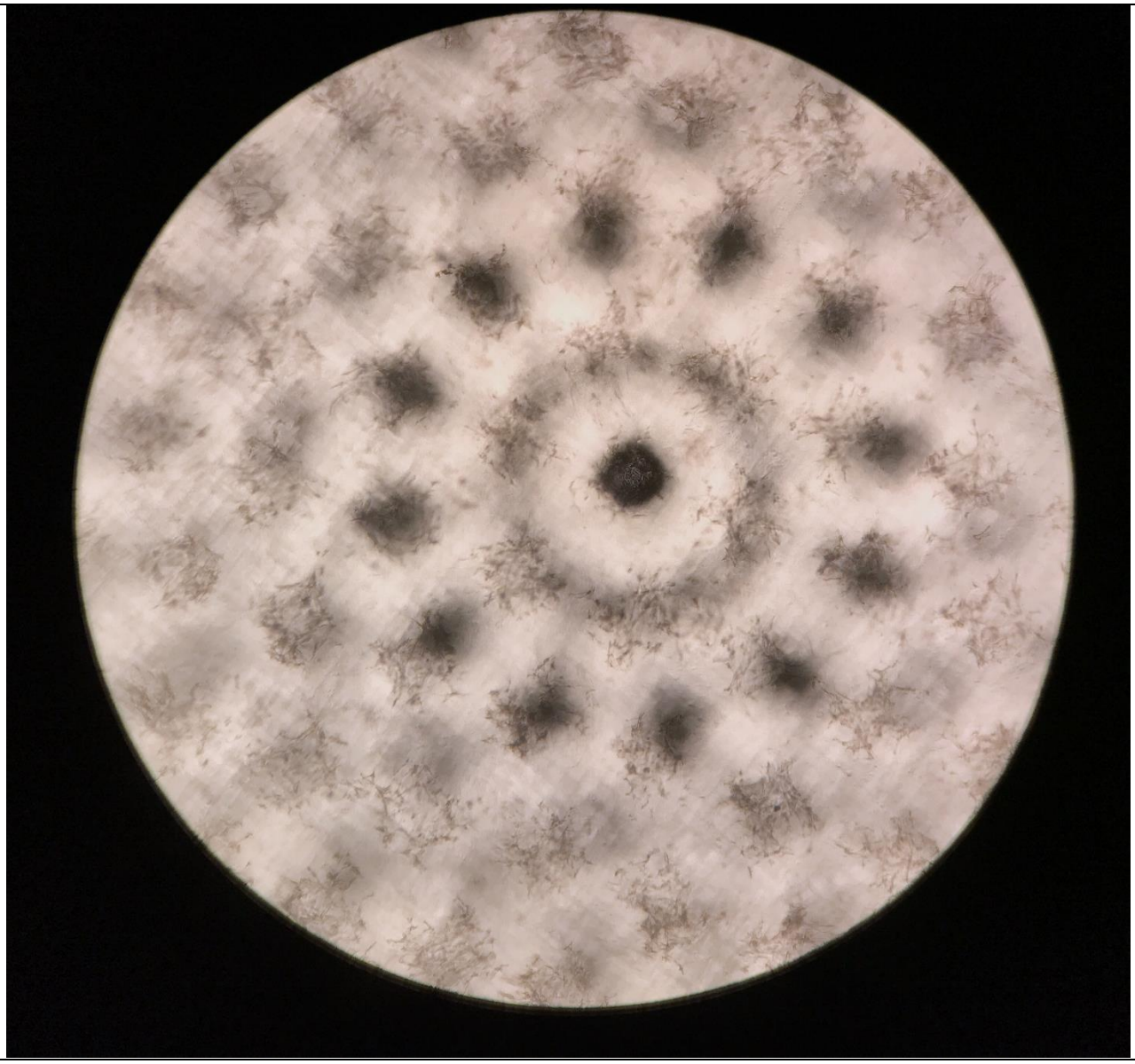
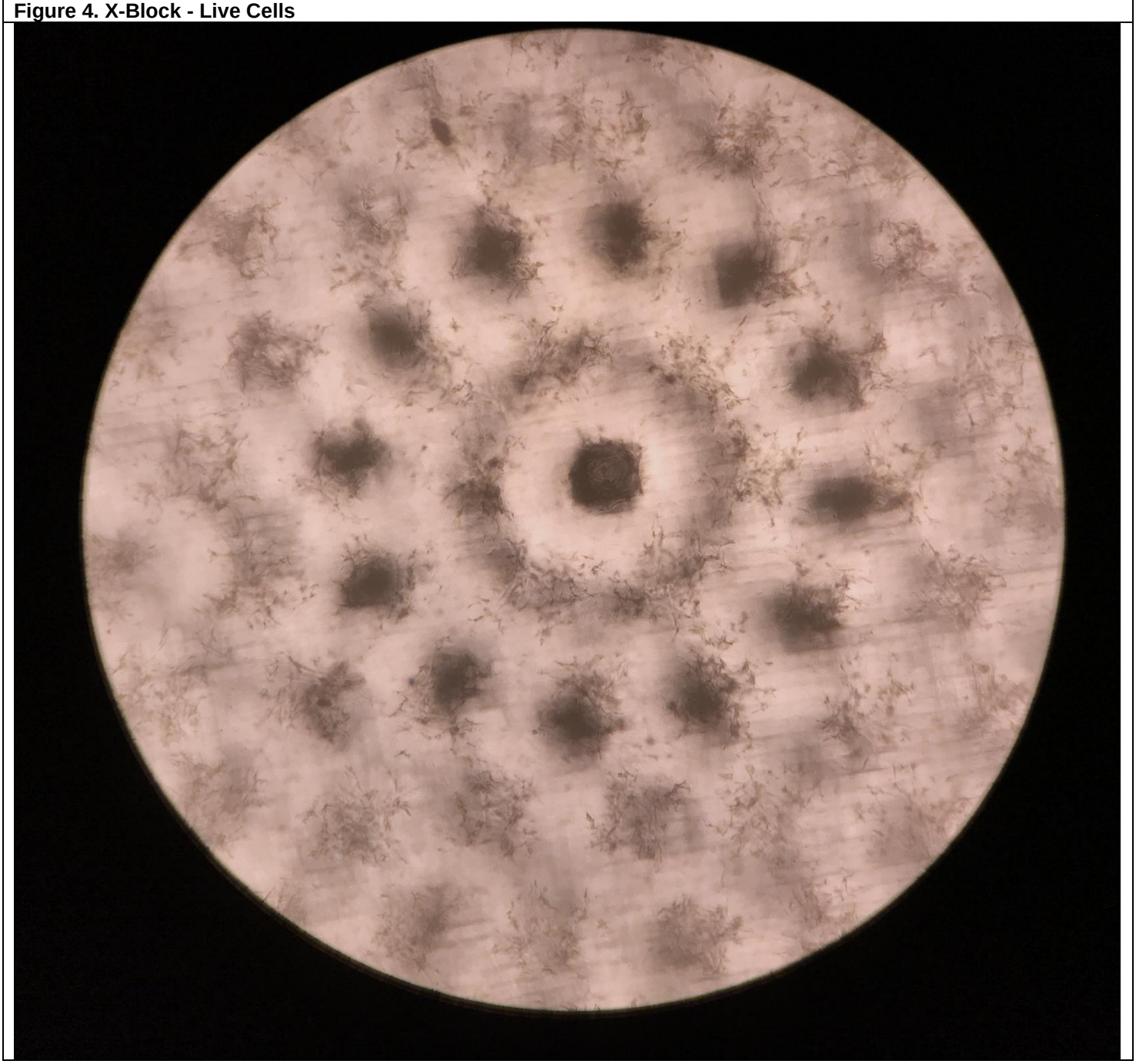


Figure 4. X-Block - Live Cells



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