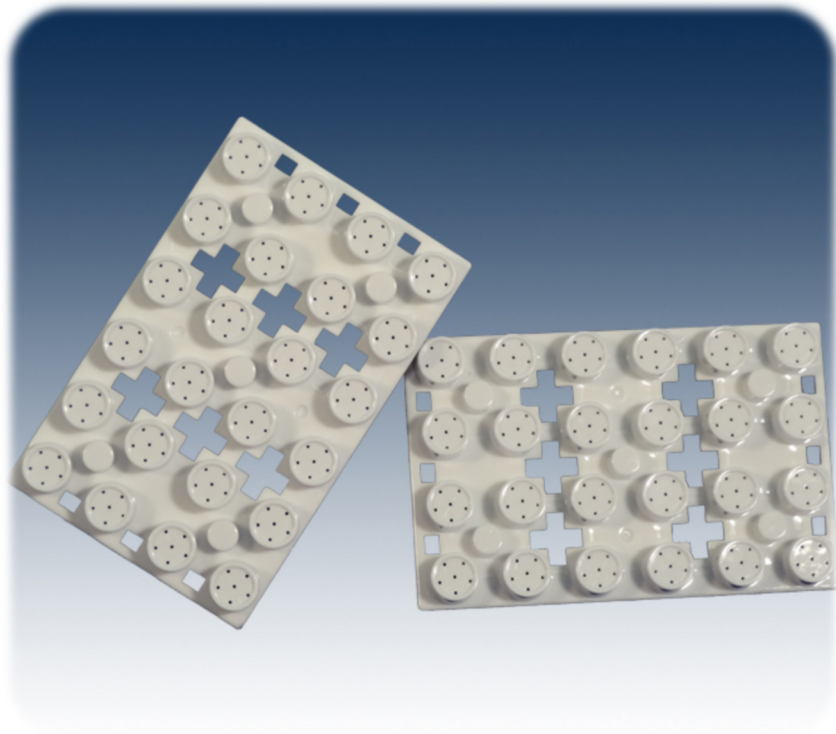




USER MANUAL

HT Cell Seeder™



05-12-17
Rev 2.1

Culturing Cells in a Mechanically Active Environment™
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INTRODUCTION

The HT Cell Seeder™ (Fig. 1) confines cells during plating to the area of the HT BioFlex® membrane that is directly over the Loading Station™. Thus, it prevents cells from being subjected to undefined strains when using the 24-well equibiaxial Loading Stations™ during strain application (Fig. 2). The HT Cell Seeder™ is inserted into the 24-well HT Baseplate (Fig. 3), and the HT BioFlex® plate plus the gasket is placed on top of the HT Cell Seeder™. The HT Cell Seeder™ is only required for seeding cells onto the membrane for a minimal period of time. Afterwards, cell feedings and experiments can be conducted as normal.

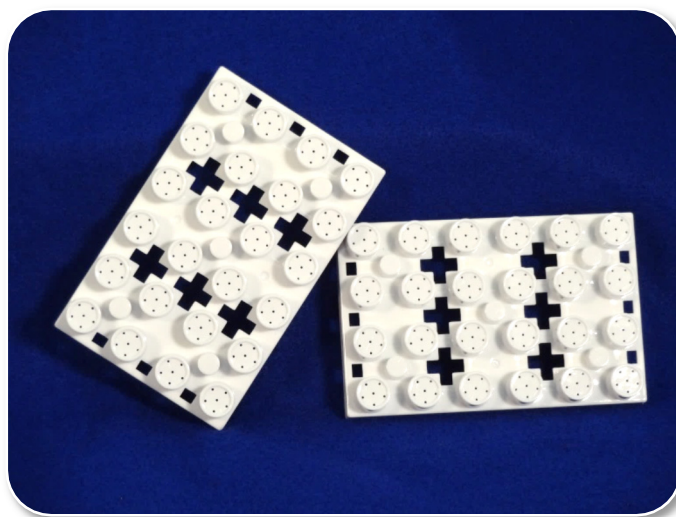


Figure 1. 24-well HT Cell Seeders™.

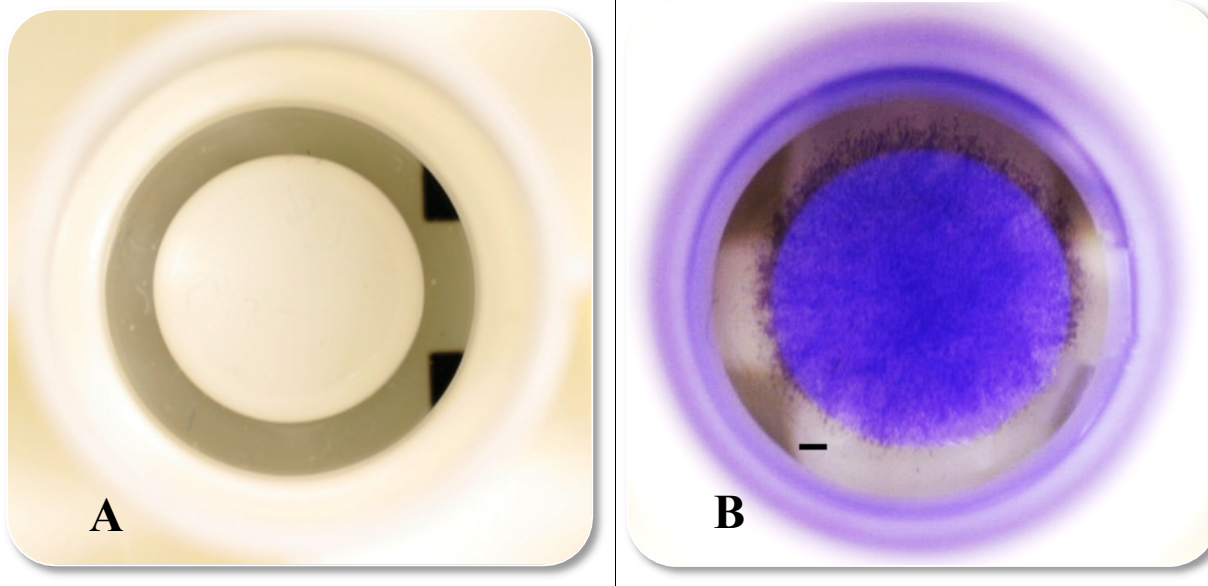


Figure 2. A) A cylindrical loading post used when applying equibiaxial strain under the well of an HT BioFlex® plate. Cells seeded on the membrane in areas outside of the loading post will be subject to undefined strains. B) The results of using an HT Cell Seeder™ when plating cells to confine them to the area directly above the loading post (blue = Crystal Violet stain; bar = 1 mm).



Figure 3. HT Cell Seeders™ in a 24-well HT Baseplate.

INSTRUCTIONS

1. Insert the HT Cell Seeder™ into a 24-well HT Baseplate, similar to how the cylindrical loading stations are placed in the baseplate.
2. Place an HT BioFlex® plate and its respective gasket over the HT Cell Seeder™, ensuring that the wells in the plate align with the posts on the HT Cell Seeder™.
3. Using the FX-5000™ Tension System, create and start a regimen with the following settings:
 - SHAPE: Static
 - MIN: 0.0
 - MAX: 8.0
 - FREQ: 1 Hz (this value must still be entered even though the regimen is static)
 - DURATION: Equal to the seeding time (see step 5 below) plus the time needed to plate the cells into the well.
 - PLATFORM: HT 24-Well Plate (Cylindrical LS)

NOTE: *The membrane may be subjected to strains up to 1-2% (10 to 20 microns per 1 millimeter) when seeding, due to the inherent strain caused when using the HT Cell Seeder™.*

4. The recommended suspension volume when using an HT Cell Seeder™ for an individual well is 175 μ L. This volume is large enough to allow uniform distribution of the media-cell suspension within the well when dispensing with a 1000 μ L micropipette (Fig. 4). Also, this volume is small enough to prevent the media-cell suspension from spilling out of the HT Cell Seeder™ when handling the baseplate.

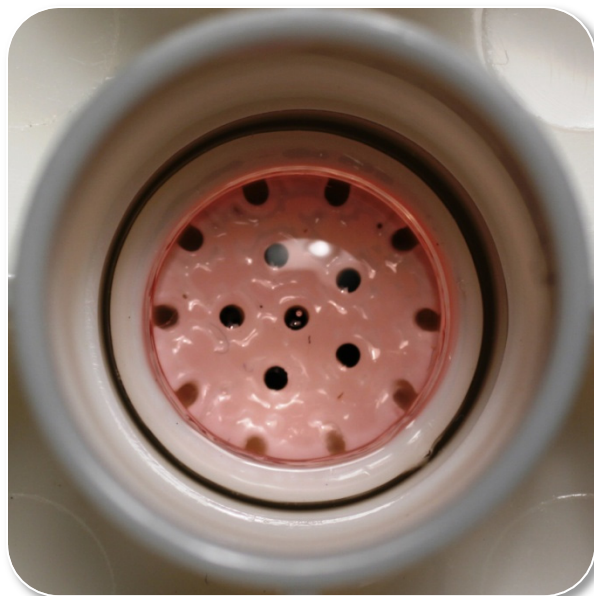


Figure 4. Suspension volume within the HT Cell Seeder™.

5. Due to the limited suspension volume, it is advised that the user try to limit the seeding time required for the cells to adhere to the membrane. We recommend a seeding time of at least two hours, but the time that will be required is dependent on the type of cells being used.
6. After the cells have adhered, remove the vacuum gradually. You can create a slow vacuum release regimen if needed that reduces the vacuum a set percent every “n” seconds. An example regimen is outlined below in Table 1.
7. Slowly add 1 mL of fresh media to each well to increase the volume for proper aspiration of non-adherent cells. Then, insert an aspirator tip near the side of the plate well (instead of the cell seeder well) and aspirate the media containing non-adherent cells.
8. Add 1 mL of fresh media to each well.

Table 1. Sample regimen parameters for releasing the vacuum pressure slowly.

Step	Shape	Min	Max	Freq	DC%	dd:hh:mm:ss	Back To	Repeat
1	Static	0.0	8.0	1.0	50.0	00:02:30:00	0	0
2	Static	0.0	6.0	1.0	50.0	00:00:00:06	0	0
3	Static	0.0	4.0	1.0	50.0	00:00:00:06	0	0
4	Static	0.0	2.0	1.0	50.0	00:00:00:06	0	0
5	Static	0.0	1.0	1.0	50.0	00:00:00:06	0	0

In this example, we are assuming a two hour seeding time plus a 30 minute set-up time (Step 1). Following seeding, this example releases the strain by 1-2% every six seconds.