



HT Cell Seeders™

Product Information Sheet

01/21/16 Rev. 1.0

The HT Cell Seeder™ confines cells during plating to the area of the HT BioFlex® membrane that is directly over the 24-well Loading Station™ (Fig. 1). Thus, it prevents cells from being subjected to undefined strains during strain application. The HT Cell Seeder™ is only required for seeding cells onto the membrane. After cell attachment, cell feedings and experiments can be conducted according to your laboratory's established protocols. For more information, see the HT Cell Seeder™ product webpage at <http://www.flexcellint.com/HTCellSeeders.htm>.

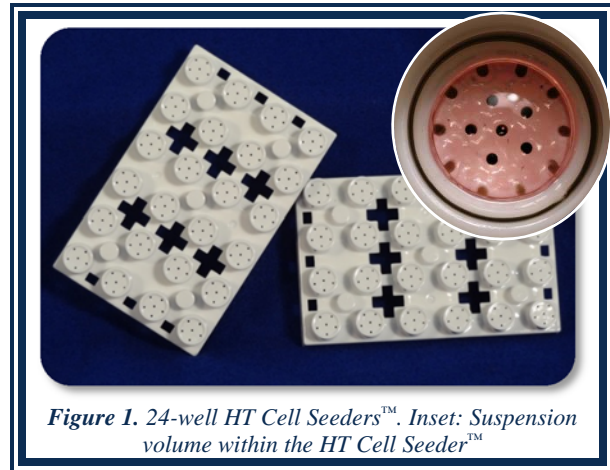


Figure 1. 24-well HT Cell Seeders™. Inset: Suspension volume within the HT Cell Seeder™

PLATING CELLS WITH A HT CELL SEEDER™

1. Insert the HT Cell Seeder™ into a 24-well HT baseplate, similar to how the cylindrical Loading Stations™ are placed into the BioFlex® 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 µl. This volume is large enough to allow uniform distribution of the media-cell suspension within the well when dispensing with a 1000 µl micropipette. Also, this volume is small enough to prevent the media-cell suspension from spilling out of the HT Cell Seeder™ when handling the baseplate.
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. Table 1 below outlines an example regimen.
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.

ORDERING INFORMATION

HT Cell Seeders™ (Cat. No. HTCS-3000) are sold in a set of four or as part of the 24-well HT BioFlex® baseplate kit (Cat. No. HTBK-4000). Baseplate kits include a baseplate, 4 gaskets, corresponding Loading Stations™, 4 cell seeders, 4 sample plates, acrylic window, grease, and software update, if needed.

Flexcell® culture plates 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.