

BioFlex® Cell Seeders

The BioFlex® Cell Seeder confines cells during plating and adhesion to the central area of the BioFlex® membrane (Fig. 1 inset) that will glide over the 25 mm Loading Station during strain (Fig. 1). Cells in this central area are subjected to well defined, equibiaxial strains. The Cell Seeder is only required for seeding cells onto the membrane during the cell adhesion process. After cell attachment, cell feedings and experiments can be conducted according to your laboratory's established protocols. For more information, see the BioFlex® Cell Seeder product webpage, which can be found at

http://www.flexcellint.com/BioFlexCellSeeders.htm.

PLATING CELLS WITH A BIOFLEX® CELL SEEDER

- 1. Insert the BioFlex® Cell Seeder into the BioFlex® baseplate, similar to how the cylindrical Loading Stations are placed into the baseplate. *Optional*: Add a thin layer of lubricant to the upper edge of the Cell Seeder to aid movement of the membrane into the Cell Seeder body.
- 2. Place a BioFlex® plate in its respective gasket over the Cell Seeder, ensuring that the wells in the plate align over the posts on the Cell Seeder.
- 3. Using the FX-5000™ Tension System, create and start a regimen with the following settings: Shape Static; Min 0.0; Max 15.0; 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 BFlx Loading Station (25mm). NOTE: The membrane may be subjected to minimal strains when seeding, due to the inherent strain caused when using the BioFlex® Cell Seeder.
- 4. The recommended suspension volume when using a BioFlex[®] Cell Seeder[™] for an individual well is 1 ml. 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 exceeding the boundary of the Cell Seeder compartment when the baseplate is moved.
- 5. Due to the limited suspension volume, it is advised that the user define the seeding time required for the cell type to adhere to the membrane. We recommend a seeding time of at least two hours, but the time required is dependent on the type of cells used.
- 6. After cells have adhered, remove the vacuum gradually. It is recommended to create a slow vacuum release regimen that reduces the vacuum a set percent every "n" seconds. Table 1 below outlines an example regimen.
- 7. Slowly add 1 ml of fresh medium to each well to increase the volume for proper aspiration of non-adherent cells. Then, insert an aspirator tip near the side of the each well (adjacent to the cell seeded area) and aspirate the media containing non-adherent cells.
- 8. Add 3 ml of fresh media per well and return the BioFlex® plate to the incubator until you are ready to apply strain.

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	15.0	1.0	50.0	00:02:30:00	0	0
2	Static	0.0	13.0	1.0	50.0	00:00:00:06	0	0
3	Static	0.0	11.0	1.0	50.0	00:00:00:06	0	0
4	Static	0.0	9.0	1.0	50.0	00:00:00:06	0	0
5	Static	0.0	7.0	1.0	50.0	00:00:00:06	0	0
6	Static	0.0	5.0	1.0	50.0	00:00:00:06	0	0
7	Static	0.0	3.0	1.0	50.0	00:00:00:06	0	0
8	Static	0.0	1.0	1.0	50.0	00:00:00:06	0	0

In this example, we show 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: BioFlex® Cell Seeders are sold individually (Cat. No. BFCS-1000) and in a set of four (Cat. No. BFCS-4000).

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.

