StagePresser™ Membrane

Product Information Sheet
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StagePresser™ membranes are 43 mm diameter silicone elastomer disks with an acrylic piston and foam sample holder (Fig. 1). StagePresser™ membranes can be used with the Flexcell® Compression System and StagePresser™ microscope device to apply an unconfined compression to cells in 3D culture or a tissue explant. When positive air pressure is applied, the silicone elastomer membrane is pushed upward compressing the sample between the piston and a stationary platen. For more information, see the StagePresser™ Membrane product webpage at http://www.flexcellint.com/StagePresserMembrane.htm.

Figure 1. *A StagePresser™ membrane with acrylic pistons adhered to the silicone elastomer well bottom and foam sample holders.*

SAMPLE PREPARATION FOR STAGEPRESSER™ MEMBRANES

In preparing a 3D culture or tissue explant sample, be sure that the sample thickness is no less than 1.0 mm and no greater than 3.81 mm (0.150"). The thickness of the compressed foam is 0.350 mm; therefore, thinner samples will not be compressed. A sample thickness of 1.0 mm or more will ensure compressibility. The maximum chamber height is 3.81 mm (0.150"); therefore, samples with a thickness greater than 3.81 mm (0.150') will be preloaded when placed into the StagePresser™ body. The sample diameter should be equal to or less than the inner diameter of the foam sample holder in the chamber, which is 5 mm (0.200"). If the user wishes to use a larger sample, they can remove the sample holder and place the sample within the larger ring that normally contains the sample holder. This will allow a sample diameter of up to 13 mm (0.525").

Three-Dimensional Cell Samples

Cells should be cultured according to your laboratory's established protocol for primary cultures or continuous cell lines in the medium of choice. In general:

1. Release cells from their substrates with 0.05% trypsin, trypsin-EDTA, 0.05% bacterial collagenase, or other means.
2. Add serum containing media to the cells to neutralize the trypsin or collagenase.
3. Count cells and determine the number of cells needed for the three-dimensional culture. Three sample methods are described below. *NOTE: Cell seeding density will vary depending on cell type. We recommend testing cell seeding densities to determine the best cell number for your application and cell type.*

Tissue Explant Samples

1. Prepare tissue according to the laboratory's established protocol.
2. If applicable, cut a 1-3 mm thick sample of the tissue explant.
3. Using a 5 mm trephine punch (or similar tool), cut out a 5 mm diameter disc of tissue.
4. Place sample in the inner ring of the sample holder as described in the next section, *Sample Placement into a StagePresser™ Device*.

Sheet Punch of a Cell-Seeded Hydrogel:

1. Prepare hydrogel (Collagel®, agarose, gelatin, alginate, etc.) according to the manufacturer's or laboratory's established protocol.
2. Suspend cells in the hydrogel at $1 \times 10^4 - 1 \times 10^5$ cells/ml.
3. Allow hydrogel to polymerize in a sheet or plug that is 1-3 mm thick.
4. Using a 5 mm trephine punch (or similar tool), cut out a 5 mm diameter disc of the cell-seeded hydrogel.
5. Place sample in the inner ring of the sample holder as described in the next section, *Sample Placement into a StagePresser™ Device*.

Direct-Placement of a Cell-Seeded Hydrogel:

1. Prepare hydrogel (Collagel®, agarose, gelatin, alginate, etc.) according to the manufacturer's or laboratory's established protocol.
2. Suspend cells in the hydrogel at $1 \times 10^4 - 1 \times 10^5$ cells/ml.

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3. Pipette cell-hydrogel suspension into the foam sample holder of the StagePresser™ membrane.
4. Allow the hydrogel to polymerize.

Pellet Culture:

1. Using primary cultures or cell line, prepare a suspension of 5 x 10^5 – 5 x 10^6 cells.
2. Spin at 10,000 x g for 5 minutes.
3. Carefully transfer cell pellet to the center of the sample holder as described in the next section, *Sample Placement into a StagePresser™ Device*.

SAMPLE PLACEMENT INTO A STAGEPRESSER™ DEVICE

1. To prepare a sample for loading, use a pair of sterile forceps to place the tissue or gel sample into the center hole of the foam sample holder in the StagePresser™ Membrane. Add 12 ml of culture medium to each culture dish.
2. Prepare all stationary platens by adjusting the center screw (Fig. 2) such that the screw bottom (part that touches the sample) is exactly flush with the bottom of the platen body. Use a pair of sterile forceps and the two holes at the adjustable center of the platen to turn the center screw.
3. Once the sample is placed within the chamber, the adjustable height top is screwed into the body of the StagePresser™, then turned clockwise until the sample just begins to contact the bottom of the glass. If you wish to determine the exact number of revolutions required, turn the adjustable top until its surface is exactly flush with the top surface of the StagePresser™ body, then see the following instructions and equations below.
   NOTE: If your sample height is larger than 3.81 mm (0.150"), turning the adjustable top to the position where its surface is flush with the StagePresser™ body will begin to compress your sample. In this case, you will want to disassemble the StagePresser™ body by removing the four screws around the periphery, then adjust the top (according to the results obtained from the following equations) after the body is removed from the base. Once the top is adjusted, reassemble the base and body with the sample already in place in the sample holder. As the body is assembled, be careful to insure that the membrane/piston assembly is accurately centered over the chamber in the base of the StagePresser™, and that the O-ring is centered over the O-ring groove around the periphery of the base.
4. Use the ***x*** value in the following equation(s) to determine the turning direction and how many revolutions will be required to begin contacting the top of your sample.
   - For sample height in millimeters, **$x = (3.81 - h_s) / 0.39$**
     where ***x*** is the number of 180 degree turns required of the adjustable platen [(+) number = clockwise turns; (-) number = counterclockwise turns] and $\boldsymbol{h_s}$ is the sample height in millimeters.
   - For sample height in inches: **$x = (0.150 - h_s) / 0.015$**
     where ***x*** is the number of 180 degree turns required of the adjustable platen [(+) number = clockwise turns; (-) number = counterclockwise turns] and $\boldsymbol{h_s}$ is the sample height in inches.
5. Use the eight medium addition holes around the periphery of the adjustable top in conjunction with the four screw holes around the periphery of the StagePresser™ body to monitor the number of revolutions or partial revolutions of the adjustable top.
6. Add medium to the cells or tissue as needed (3-5 ml) through the holes around the periphery of the adjustable top.
7. The base of the assembly should fit onto your microscope base for proper viewing. A larger base is also included should you wish to screw the device onto your microscope. Simply remove the four screws in the bottom of the small base and reassemble the device with the larger base.
8. To view your tissue sample or gel under a microscope, a lighting source from above will be required. Direct the source to the sample and adjust as necessary to illuminate the sample for viewing with your microscope. An upright or standard microscope is required for viewing the 3D sample.

ORDERING INFORMATION

StagePresser™ membranes (Cat. No. SPM-3000) are sold individually. Each plate is sterile and individually packaged in a standard culture dish. Flexcell® membranes have a shelf life of 1 year when stored at room temperature or 4 °C in the dark or out of direct light.