

# Preparing The Flow Cell System

The fully assembled flow cell apparatus you have purchased is sterilized by gamma radiation, so no sterilization procedure is necessary for its use. It is ready to attach to your sterilized medium container and delivery tubing.

The exposed manifold barbed fitting should be wiped with alcohol before inserting into the delivery tubing from the medium container as a precaution against possible bacterial transfer when handling. The barbed fitting is for 1/8" inner diameter (ID) tubing.

*This apparatus is designed for a single use.*

**None of the elements can be autoclaved.**

## Procedure For Filling The Flow System

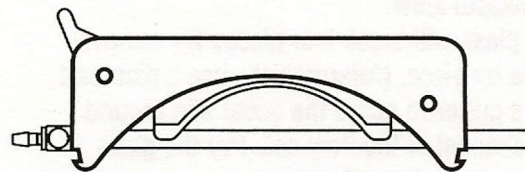


FIGURE A

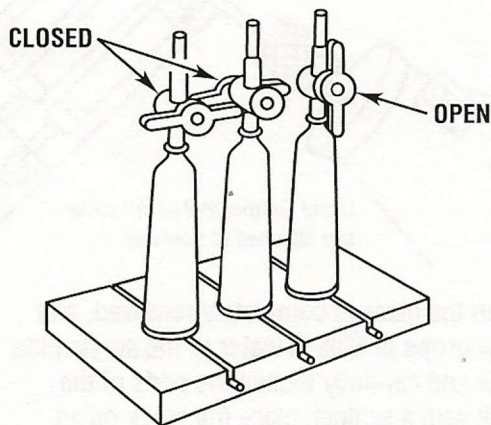


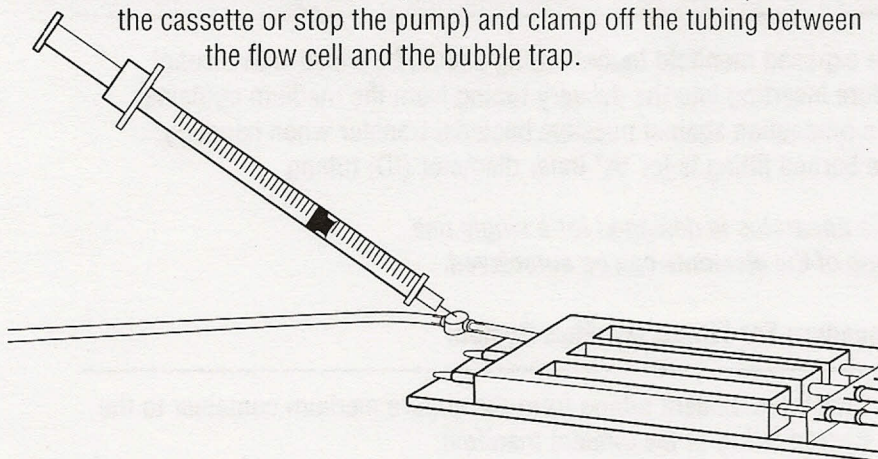
FIGURE B

- 1 Attach the influent tubing from the culture medium container to the barbed fitting of the influent manifold.
- 2 Insert the pump tubing into the tubing cassette of the peristaltic pump according to **figure A**. DO NOT twist the tubing, otherwise tube life and delivery volume will be reduced.
- 3 Install the loaded cassettes into the pump and attach the effluent manifold to the tubing going to the waste container.
- 4 The medium and effluent bottles must be placed at or above the level of the flow cell for effective flow control.
- 5 Before turning the pump on, make sure the bubble trap stopcocks are in the closed position. (**See figure B**)
- 6 Turn the pump on and press Max button to prime the system with culture medium. When the culture fluid begins to flow into the effluent bottle, slow the pump to about 10% value. Turn the stopcocks, one by one, to the open position and allow culture fluid to fill about 1/2 the cylinder; then turn the stopcock off. The captured air in the tops of the cylinder exerts a small positive pressure on the flowing medium.
- 7 Make sure that all air bubbles are out of the system by observation, and carefully tapping the flow cells while the pump is running.
- 8 **Determining Flow Volume.**  
Detach the effluent tube from the waste bottle and allow medium to flow into a 100 ml graduated cylinder for 10 minutes to determine the flow rate. For example, at 4% rate (i.e. .44 rpm on the Ismatech pump) 10 ml may collect in 10 minutes. This makes the flow rate 1 ml/minute from the three channels of the flow cell, or .33 ml/minute per channel. Adjust the rpm up or down to achieve the desired flow rate. The flow rate varies with the number of pump rollers (4/6/8) and the ID of the pump tubing. The tubing ID on the Stovall product is 1.14 mm. Pump instruction books give nominal rates for different tubing interior diameters.



## Inoculation Of Flow Channels

- 1 Before each inoculation, stop the flow of culture medium (i.e. release the cassette or stop the pump) and clamp off the tubing between the flow cell and the bubble trap.



- 2 Swab with 96% ethanol the injection ports/septums which align with each channel of the flow cell.
- 3 Fill a .5 ml needle syringe with a diluted, overnight culture of cells.
- 4 Insert the syringe needle into the injection port/septum and inject the cells into the port and channel.
- 5 Start the flow of culture medium for 2 or 3 seconds to carry the cells into the flow cell channel. Observe the presence of inoculant in the channel.
- 6 To allow the cells to establish on the glass surface of the flow cell, turn the flow cell upside down with the glass surface downward.
- 7 After 1 hour, turn the flow cell upright, loosen the clamp(s) and start the medium flow.
- 8 Observe the bubble trap periodically for release of bubbles and maintain the level of medium at approximately  $\frac{1}{2}$  the cylinder height by releasing air through the stopcock.

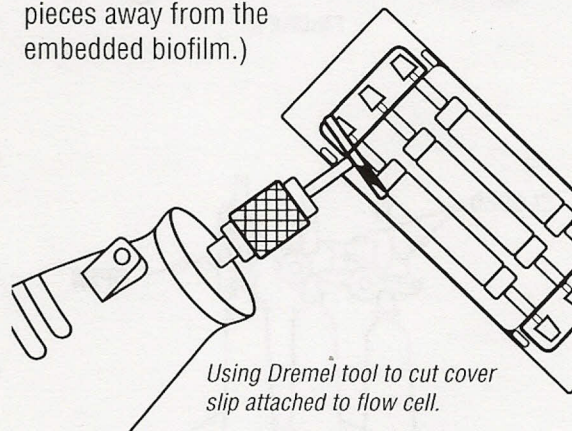
For methods and techniques concerning *Fixation of Biofilms*, *Embedding of Biofilms* and *Hybridization of Embedded Biofilms*, refer to Volume 310 of *Methods in Enzymology on Biofilms*, edited by Ron J. Doyle, published by Academic Press, 1999.

## Removing Embedded Biofilm From The Flow Channels

Cutting and removing the glass from the flow cell channels to access the embedded biofilm is a bit tricky. The cleanest and most controllable technique is to use a Dremel tool with a thin cut off wheel to:

- (1) Cut the glass cover slip on the four sides of the flow channel.
- (2) Pry the glass completely away from the embedded biofilm using a scalpel or exacto knife.

The glass will break into pieces for removal piece by piece. (Alternatively, use a diamond glass cutter to score the cover slip around the channel of the flow cell. Pry the glass pieces away from the embedded biofilm.)



When the glass is completely removed, add a few drops of Milli-Q water to the acrylamide block and carefully loosen the ends of the block with a scalpel, place the block on an object glass and cover it with 1 ml of Milli-Q water. Make sure that the side that faced the cover slip in the flow cell is turned downward. Put the object glass with biofilm block into a petri dish and seal the petri dish with para film. Note the original direction of flow. Store at 4° C.

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