FC 280 / 281 / 284 / 285
Operator's Manual

The models FC 280 / 281 / 284 / 285 flow cell designs are based on the FC 81 but accommodate two growth channels, rather than one. This design allows biofilms to be grown side by side for duplicate or "control – treatment" experiments. Biofilms growing in each channel can be easily compared microscopically by simply moving the microscope stage backwards or forwards. The body of the flow cell consists of a polycarbonate or anodized aluminum flow channel. Glass viewing ports allow transmission microscopy of biofilm growth in the flow channel.

FC 280-PC

Viewing Windows

The viewing windows on the bottom of the reactor consist of a glass microscope slide, and on the top of the reactor a no. 2, 24mm x 60mm cover slip. The top and bottom designation is arbitrary. The flow cell may be operated in any orientation. Milled recesses on the flow channel accept the glass slide or glass cover slip. The recess for the glass slide is deeper than the recess for the cover slip.

The glass viewing windows are held in place by a pair of anodized aluminum cover plates. The cover plates also compress the silicone rubber gasket material to provide a leak-proof seal.

The flow chambers on the flow cell are approximately 2.0" (50 mm) long by 0.5" (13 mm) wide by 0.10" (2.35 mm) deep. The flow chambers can also be used to insert irregularly shaped materials, such as sutures, pieces of catheter, sand, etc. for imaging biofilm growth.

Injection ports

The FC 281 and 285 flow cells come equipped with two autoclavable injection ports which may be used for inoculation, the addition of microscopy stains, or chemicals such as antimicrobial agents, etc.

1. Flow Cell Assembly and Operation

It is very important that the flow cells be assembled properly to provide a leak-proof seal. The flow cell is designed for a **maximum flow rate of 3.5 mL/minute**. Flow rates in excess of 3.5mL/minute could damage the flow cell or cause leaks.

Disassembly

1. Remove the seventeen (17) screws holding the cover plates in place.
2. Remove the cover plate (most likely the gaskets and glass viewing ports will remain with the flow cell).
3 Carefully remove the glass cover slip from the gasket material. If the cover slip is broken, remove and discard the cover slip.
4. Carefully clean the gasket surface, removing all glass and other debris. **It is very important no residual glass or other material be left on the gasket surface.**
5. Clean the flow channel surfaces using water and laboratory detergent. **Do not scrub or mar the glass/flow channel mating surfaces.**

Assembly

1. Carefully position the glass slide in the recessed slot on the flow channel (the deeper and longer of the recessed slots).
2. Carefully position the silicone gasket on the inside surface of the cover plate with threaded screw holes (no recessed beveling of the screw holes). Align the holes in the gasket to the threaded holes on the cover plate (the silicone gasket will stick to the anodized aluminum plate if it is wet).
3. Carefully position the cover plate/gasket onto the flow channel. Make sure the glass slide stays in the recessed slot and the screw holes in the gasket still line up with the holes in the cover plate. Insert short screws into the corner beveled recess and tighten the screws to seal the flow channel component to the base mount plate (if using an inverted microscope, the cover slip side of the flow channel will be used, and the cover slip substituted for the microscope slide).
4. With the stage mount plate mounted on the flow channel, invert the flow channel, and set it down on a clean, flat, stable surface.
5. Carefully position a clean, unbroken cover slip in the shallow recessed slot on the flow channel. It is very important the cover slip be properly positioned in the recessed slot. **Improper placement will result in cover slip breakage upon tightening the cover plates.**
6. Carefully position the other clean gasket on the other cover plate (plate with recessed beveled screw holes; positioning is aided by wetting the gasket). Align the screw holes in the gasket and the cover plate.
7. Put several screws through the beveled holes on the cover plate (center) and through the holes on the gasket. These screws will help hold the gasket in place as it is placed onto the flow channel and align the cover plate onto the flow channel.

8. Carefully place the cover plate with gasket onto the flow channel. **It is very important the cover slip remain properly positioned in the recessed slot (improper placement will result in cover slip breakage and or leaking). [**Screws inserted into the cover plate while installing the cover plate will help align the plate as it is lowered onto the cover slip and flow channel. Do not move the cover plate once it has contacted the cover slip, as the cover slip will move out of the channel, resulting in breakage upon tightening the screws.]
9. Place the screws into the beveled screw holes and tighten evenly around the perimeter, alternating sides as the screws are tightened.
   It is important the screw holes in the cover plate align with the holes in the gasket material. Improper alignment may result in leakage.
10. Install tubing on the tube ports and pump liquid through the flow cell to confirm a liquid tight seal.

CAUTION: To avoid damaging flow cell, do not overtighten metal fittings and screws in polycarbonate models. Compression O-rings on fittings do not require excessive force to seal.

2. Autoclaving Instructions

The FC 280 series Flow Cell is autoclavable up to 121°C, at 20 minutes. Using temperatures or times past what is suggested may cause unnecessary stress to some materials and will not be covered under warranty. Polycarbonate plastic will degrade with a numerous autoclave cycles and may eventually require replacement.

BioSurface Technologies recommends always using the slow (liquid) exhaust option. The screws holding the cover plates on the flow cell should be loosened to allow for material expansion when autoclaving the polycarbonate flow cell. Tubing connected to the flow cell should allow free exchange of steam with the surrounding environment. BST recommends using a gas permeable material to cover the tubing ends such as autoclave paper instead of a gas-impermeable material, such as tin foil (for the polycarbonate flow cell, slowly tighten the screws as the flow cell cools or wait until the flow cell is completely cooled before tightening the screws). Align all tubing connected to the flow cell to minimize stress on the flow cell fittings.

3. Spare Parts

Included with the flow cell:

- Spare screws (4-40, stainless steel)
- Spare gaskets
- Additional cover slips (no. 2, 24mm x 60mm)
- Glass slide (for viewing window, 1mm thick x 25mm x 75mm)

With proper care, the gaskets should be re-useable for numerous experimental evaluations. The spare gaskets provided require removal of the clear protective polymer sheeting from both sides of the gasket prior to installation. The gasket material is 0.020 inches thick silicone rubber. Other materials may be used.

4. Troubleshooting

Problem: Glass viewing ports keep breaking
Solution:

- Check to ensure the glass is properly positioned in the recessed slot.
- Ensure the gasket surfaces and flow channel mating surfaces are total free of debris.

Problem: Flow cell leaks
Solution:

- Tighten screws further to compress gasket.
- Make sure flow channel and hose barbs are free and clear of obstructions.
- Make sure glass viewing ports are not cracked or broken.
- Check gasket for rips or tears. Clear of all debris. Replace as necessary.
- High flow rates may create too much back pressure to contain leakage. Reduce flow rate. **The flow cell is designed for a maximum flow rate of 3.5 ml/minute.**
- Glass slide well depth is machined to a precise depth to accommodate 1.00 mm thickness glass slides. A tolerance of +/- 0.02 mm glass slide thickness variation is acceptable. If the flow cell is leaking from the glass slide side of the flow cell, try a thicker glass slide to generate a seal.

5. Flow Cell Ancillary Equipment List

NOTE: *BioSurface Technologies does not sell or supply the ancillary equipment described below. This or comparable equipment is required to complete the reactor system set-up. Please check with your local vendors for availability and current pricing. Equipment listed is not recommended equipment, but an aid to help you identify compatible equipment.*

Pump: There are many types of peristaltic pumps available and you may opt for alternative types and pump head configurations (multi-channel versus single channel, higher/lower rpm range, etc.). Depending on what you need to add to the reactor during operation, you may require more than 1 pump or a multi-head pump (media for growth and biofilm treatment chemical for some duration that may require an additional pump).

- **L/S Variable Speed Modular Drive Pump system (1-100 RPM)**
  (Cole-Parmer P/N EW-07557-10; 90-230 VAC CE certified)
- **Ismatec Minicartridge Pump Heads for Masterflex L/S Drives**
  (Cole-Parmer P/N EW-07623-10)
    - Has 8 channels; will accommodate 8 influent lines. Alternate pump heads are available for 2 or 4 channel systems.
    - Accepts size 13 tubing.

Tubing and Fittings: The tubing you choose depends on the chemical compatibility, gas permeability, wear resistance in peristaltic pumps, and pricing. You must choose the tubing that best fits your needs. C-Flex tubing (listed below) is similar to silicone tubing but has a low gas-permeability compared to silicone. If gas-permeability is not an issue, standard silicone tubing is acceptable.

- **Size 13 tubing 25' C-Flex:**
  (Cole Parmer P/N HV-06424-13)
    - For use with the Ismatec pump head cartridges, connected to the size 16 tubing using adapters.
- **Size 13/16 tubing adapters** (connect size 13 to size 16 tubing)
  (Cole Parmer P/N EW-30622-22)
- **Masterflex, C-Flex (50 A), L/S 16, 25 ft.** (Cole Parmer P/N EW-06424-16)
    - Accepts the 1/8" barbed connectors (3.2 mm connectors).
    - To connect to the media supply reservoir and to the flow cell connections.
- **Male Luer Lock x 1/8" barb –Polypropylene (included with standard flow cell system** (Cole Parmer P/N A-30504-10)
- **Masterflex, C-Flex (50 A), ¼" ID x 7/16" OD, 25 ft**
  (Cole Parmer P/N EW-06424-72)
    - A few lengths and adapters to get from the carboy to the smaller diameter tubing, and as a siphon tube inside the carboy.
- **Nylon 1/4" x 1/8" adapter** (Cole Parmer P/N EW-30622-28)
    - Needed to get from the 3/16" or 1/4" ID to the 1/8" tubing.

Carboy: The carboy should be selected based on experiment needs and may be larger or smaller than what is suggested below. Ported lids can be purchased from suppliers, but standard lids are easily converted to ported lids using the following fittings or similar.

- **Cole-Parmer Heavy-Duty PP Carboy with Shoulder Handle, 10 L**
  (Cole Parmer P/N EW-62507-10)

- **Filling / Venting ports for carboy lid - 1/4” tubing**
  (Cole Parmer P/N EW-06259-10)
  - Each reservoir vessel will require this port for the bacterial air vent.
- **Filling / Venting ports for carboy lid - 1/2” tubing**
  (Cole Parmer P/N EW-06259-00)
- **Bacterial vent for media and waste reservoirs**
  Any 0.22-0.45 um filter will work; can use pre-sterilized filters and mount on the vessels immediately after autoclaving.

Suggested Ancillary Equipment Suppliers:

Cole Parmer: 800-323-4340 (www.coleparmer.com)
Fisher Scientific: 1 800-766-7000 (www.fishersci.com)