BioFocus® Cartridge Assembly Kit

Catalog 148-3050

For use with 350–375 µm OD capillaries

Section 1
General Information

Introduction

The Cartridge Assembly Kit comes with a 50 cm x 50 µ ID uncoated capillary installed. Please check the contents of the kit against the packing list on page 7 and read all instructions before beginning disassembly or assembly of the cartridge. Refer to the drawings on pages 9 and 10 for identification of items contained in the kit.

These instructions are divided into three sections, **Disassembly**, **Assembly**, and **Selecting Initial Operating Conditions.**

Disassembly

Please refer to the diagram on page 9 when assembling or disassembling the cartridge.

1. Remove the cartridge over by sliding it up the cartridge body. Allow the polyurethane tubing containing the capillary to uncoil.
2. Pull the inlet barb fitting and capillary out of the inlet barb port. This is a luer type fitting and does not require unscrewing.
3. Remove the optical bench lock by unscrewing it with your fingers.
4. Remove the retainer wedge from the optical bench by it sliding to the right.

5. Twist outlet barb fitting so that wings become vertical and allow it to be pulled straight out of the outlet barb port. Continue this motion until the capillary is pulled completely from the cartridge body. This must be done carefully to prevent the capillary detectionwindow from being broken. Save the red silicone seal for reuse unless it is damaged. The optical bench need not be removed unless it is to be replaced.
6. Slide the installation tool over the end of the inlet seal fitting and remove it by unscrewing. A red o-ring and a red silicone seal might remain inside the thread hole that the inlet seal fitting was removed from. Use a short length of capillary to carefully pick these parts out of the cartridge body for reuse if not damaged.
7. If the polyurethane tubing is to be used with a new piece of capillary, pull the old capillary out. It may be necessary to straighten the tubing by taping the ends to the table surface to facilitate removal of the capillary.

Assembly

1. If necessary, clean inlet seal, inlet and outlet barb fittings, optical bench, retainer wedge and optical bench lock with high grade ethanol. The O-rings on the inlet seal and outlet barb fitting are lubricated with a thin film of vacuum grease and should be regreased after being cleaned with methanol (see step 16). Wash the optical bench and retaining wedges separately from the other parts to avoid contamination with vacuum grease residue. Allow parts to air dry, or use clean, dry compressed nitrogen. After cleaning, insert the optical bench into the optical bench seat from the front with the slit accessible from the front of the cartridge and oriented to the right. The optical bench must be fully inserted into the front of the cartridge body; the square flange should be in contact with the seating surface.

2. Cut an appropriate length of polyurethane tubing (total finished capillary length minus 13.9 cm; refer to column four in Table 1 on page 5.). Flare both ends of the tubing with the flare tip of the alignment tool while grasping the tubing with the small piece of sandpaper provided. Insert the outlet barb fitting into one end of the tubing and the inlet barb fitting into the other end. Make sure that the barb tapers are fully inserted so that the end of the tubing completely covers the barb.

   To facilitate the insertion of the capillary, lay the tubing straight on the working surface and tape each end down. An alternative to taping the tubing to the working surface is to use the PTFE guide tubing to facilitate the insertion of the capillary (see step 4).

3. The capillary is cut using the white ceramic tile included in the kit. Hold the tile at about a 45 degree angle to the capillary and lightly score the surface with a single stroke. The goal is to cut through the polyimide cladding without completely severing the capillary. Gently bend the capillary to break it at the scored point.

   For a capillary without a detection window, cut a working length of capillary equal to the desired finished total length plus 4 cm excess. See samples in Table 1. This allows 2 cm excess capillary at each end to facilitate assembly.

   If the capillary already has a detection window made, cut the outlet end 6.6 cm from the detection window. Now measure the working length (total finished length plus 4 cm excess) from the outlet end and cut the inlet end. The outlet end is the closest to the detection window. Avoid touching the detection window; small amounts of finger oils or other contaminants will cause baseline drift.

4. Insert the capillary inlet end into the flat side of a red silicone seal. Push the silicone seal up to the detection window, but not over it. For short or medium lengths the capillary may be inserted directly into the polyurethane tubing. Insert the inlet end of the capillary into the outlet barb fitting and through the polyurethane tubing until the red silicone seal reaches the outlet barb fitting. If the capillary sticks during insertion, rotating it while inserting may help.

   As an alternative to taping the tubing to the working surface and for long capillary lengths the PTFE capillary guide tube may be used to facilitate insertion of the capillary. The PTFE guide tubing is intended to be reused and should not be cut shorter than the original one meter length.

   The PTFE guide tube is inserted into the polyurethane tubing from the inlet barb fitting end all the way to the outlet barb fitting. As stated above, do not cut off excess PTFE tubing extending from the inlet barb fitting. Next, thread the inlet end of the capillary through the outlet barb fitting and into the PTFE guide tube (inside the polyurethane tube). The capillary should now easily slide into the PTFE tubing until the red silicone seal reaches the outlet barb fitting. While holding the outlet end of the capillary in place, remove the PTFE guide tube by pulling it from the inlet end.

5. Untape the outlet end of the tubing. If the capillary is short, it may be easier to untape both ends at this point.

6. If there is no detection window, remove the polyimide cladding from a 0.6 cm section of the capillary 6.6 cm from the outlet end. The cladding can be removed using heat or acid; contact the capillary manufacturer for details. Clean and dry the detection window with ethanol.

7. Make sure that the O-rings on the outlet barb have a thin film of vacuum grease on them. While holding the outlet barb fitting, use the outlet guide at the top of the cartridge to carefully thread the outlet end of the capillary into the outlet fitting port, through the cartridge body and optical bench. Insert the outlet barb fitting into the outlet barb port with the wings oriented so that it becomes fully seated. Twist the fitting one quarter turn to lock the wings into place.

   Carefully pull the capillary from either the inlet or outlet side of the polyurethane tubing to center the detection window in the middle of the optical bench. If the capillary sticks, straighten the polyurethane tubing and tape it to the working surface. The capillary will slide through a straightened polyurethane tube.

8. Insert the thin side of the retainer wedge (with the raised dot facing up) into the slit in the optical bench and firmly press into place. Inspect the optical path for any gaps between the capillary and the retainer wedge or optical bench.

9. Thread the optical bench lock onto the outlet end of the capillary. While pressing the optical bench into the front of the cartridge body, screw in the optical bench lock untilb finger-tight. IMPORTANT: do not use a tool to tighten this part; over tightening can damage the optical bench and cartridge body.

10. Using the inlet guide at the top of the cartridge, thread the inlet end of the capillary through the inlet port, then press the inlet barb fitting firmly into the luer fitting of the inlet port.

11. Thread a red silicone seal onto the inlet end of the capillary with the flat side of the seal facing the cartridge.

12. The inlet seal fitting can be screwed in with fingers only or by using the installation tool. Either end of the tool may be used to fit over the inlet seal fitting. Thread the inlet seal onto the end of the capillary and screw it into the cartridge until snug. DO NOT OVERTIGHTEN. Doing so may damage the silicone seal which will compromise the performance of the cartridge at high voltages. Use ethanol to clean the exposed portion of the capillary.

    When disassembling the cartridge, the small silicone seal and a red o-ring might be retained inside the cartridge. The o-ring should be carefully removed and put back on the inlet seal before reassembling. The silicone seal can be left in place unless it has been damaged and is causing current leakage. When removing either part, care must be taken not to damage the threads inside the cartridge body. The silicone seal can be safely removed using a short length of capillary as a tool to pick it out.

13. Coil the polyurethane tubing inside the cartridge body. In order for the cartridge to fit into the BioFocus cartridge holder, the coil should not be more than two layers deep. The cartridge body can accommodate sufficient tubing for capillaries up to 100 cm total length. Slide the cartridge cover into the front of the cartridge body to hold the tubing in place.

14. Insert the capillary outlet in the alignment tool so that the bottom of the cartridge is flush with the end of the alignment tool. Gently draw the white ceramic square through the alignment tool's 45 degree cutting guide to score the capillary. Use the thumb and forefinger to quickly flick the excess capillary away. This technique produces a clean, square end to the capillary. An uneven or ragged cut can cause reproducibility errors due to sample or buffer carry over. Repeat the procedure for the capillary inlet.

If a gel-filled capillary is being installed in the cartridge, the ends of the capillary must not be allowed to dry out. Follow the manufacturer's instructions on how to cut the capillary and prevent drying.

15. Use the BioFocus Cartridge Coolant Purge device filled with distilled water to check for leaks around the fittings. See the BioFocus instruction manual for direction on the use of this device. Locate the source of any leaks and tighten the fitting or connection.

16. Also, on the BioFocus instrument, check the hole on the left side of the bottom of the cartridge holder where the inlet end of the capillary will be inserted. This is a high voltage area and it is critical that it be kept clean of buffer residue, dirt and dust. Contamination or damage in this area may cause current leakage, arcing and baseline noise at high voltages.

    If significant spiking of the baseline occurs, it may be due to contaminants in the high voltage area of the cartridge holder. The inlet electrode housing should be removed and carefully cleaned. Care must be taken to avoid bending the electrode. Follow directions in the BioFocus manual. Also, the outside of the white vial holders of the inlet carousel should be cleaned periodically.

17. Place PTFE capillary covers over the inlet and outlet ends of the capillary. Make sure that the cover is pushed over the small diameter portion of the inlet seal and optical bench lock. To protect the capillary, these covers should be kept in place during installation of the cartridge into the BioFocus instrument. On older BioFocus instruments it may be easier to guide the cartridge into place by removing the front plate of the cartridge holder first. On BioFocus 2000 and newer BioFocus 3000 instruments, push in and rotate the cone shaped cover release buttons that are just above the front plate. The flat spots should be horizontal and the buttons will remain retracted. Insertion is easiest on newer instruments with the front plate in place. Lock the cartridge into place.

    Important: The PTFE capillary covers must be removed from beneath via the carousel compartment prior to running an automation sequence or diagnostic test. Remove the carousels first to facilitate removal of the PTFE covers.

    Store the cartridge with the PTFE capillary covers to protect the capillary ends. If the capillary is gel-filled, the ends must be prevented from drying out. A small amount of buffer or distilled water can be injected in the PTFE cover before placing it on the capillary, or follow the recommendations of the capillary manufacturer.

Table 1. Capillary Length Calculations

Finished Total Length (**FL** = Inlet to Outlet) centimeters	Finished Effective Length (**EL** = Inlet to Detector = FL-4.6 cm)	Working Capillary Length (**WL** = FL+4 cm)	Polyurethane Tubing Length (**TL** = FL - 13.9 cm)
24	19.4	28	10.1
30	25.4	34	16.1
36	31.4	40	22.1
40	35.4	44	26.1
50	45.4	54	36.1
60	55.4	64	46.1
75	70.4	79	61.1
100	95.4	104	86.1

Selecting Initial Operating Conditions

This section is meant to be a guide to selecting initial operating conditions for any method that does not use a gel-filled capillary. For gel-filled capillaries, please follow the guidelines of the manufacturer. Further optimization of operating conditions may be necessary.

This protocol assumes that the user has basic familiarity with the operation of the BioFocus System. For an introduction or review of operating procedures and the terminology, the user is referred to the system instruction manual.

Application of high voltage causes Joule heating within the capillary. Excess heat may cause a decrease in the resolution and efficiency of the separation. Three factors are directly proportional to the amount of Joule heat generation in the capillary:

1. the conductivity of the buffer
2. the cross sectional area of the capillary

3. the voltage applied

These three factors are adjusted to optimize any given separation. Please refer to the BioFocus Instruction manual for a discussion of the consideration of each of these factors.

The BioFocus capillary cartridge is designed to remove Joule heat with a liquid coolant flowing continuously at high velocity around the capillary inside the polyurethane coolant tubing (see illustration of cartridge parts). In general, a power level of 0.05 watts/cm of capillary is considered a safe starting point for a fist separation. To determine a set of starting parameters, the following procedure is recommended.

1. Determine the full length of the capillary. After proper assembly of the cartridge, insert it into the BioFocus instrument.
2. Fill two microfuge vials with the buffer you plan to use.
3. Place the two vials in positions 1 of the inlet and outlet carousels.
4. Under the **Commands** pull down menu, enter the **Diagnostic** menu, then the **Pressure** screen. Set **SetupTest** to **HIGH PRESSURE MODE, INLET VIAL** to 1, **OUTLET VIAL** to 1 and **MAXIMUM LIMIT FOR TESTING** to 30 seconds. Press **START**.
5. Immediately after the capillary has been filled with buffer, exit the Pressure Diagnostics and enter the Electrical Diagnostics.
6. Enter a voltage of 10 kV and a current of 300 µA. Set **Select Test** to **RUN** and **Select Mode** to **CONSTANT VOLTAGE**.
7. Run the diagnostic and make note of the current.

With **L** as the capillary length in cm, **i** as the current in µA and **V** as the voltage in kV, determine the product $\mathbf{P_L}$ (power per length) below.

$$\mathbf{P_L = \frac{i \times V}{1000 \times L}}$$

If $\mathbf{P_L}$ is less than 0.05 watts/cm, the voltage can be increased until $\mathbf{P_L}$ = 0.05 watts/cm. If $\mathbf{P_L}$ is greater than 0.05 watts/cm, you should consider reducing the voltage. Alternatively, capillary length, capillary inside diameter and buffer conductivity can also be adjusted to keep the $\mathbf{P_L}$ below the 0.05 watts/cm guideline.

As an example, suppose you wish to perform a separation in a 40 cm capillary. After following steps 1–7 above, the current is observed to be 120 µA at 10 kV. This gives $\mathbf{P_L}$ = 0.03 watts/cm. This is well within the safe limits of heat generation. To increase the speed of the separation, you may want to increase the voltage and run the Electrical Diagnostic again. If the voltage is increased to a point where $\mathbf{P_L}$ exceeds 0.05 watts/cm, thermal distortions may begin to occur in the sample bands, decreasing resolution.

BioFocus Cartridge Assembly Kit
148-3050
Packing List

Assembled cartridge includes

Quantity	Description	Quantity	Description
1	Cartridge Body	1	Polyurethane Tubing
1	Cartridge Cover	1	Outlet Barb
1	Capillary, 50 cm x 50 µm ID x 375 µm Od, uncoated	1	Optical Bench
1	Inlet Seal Fitting	1	Retainer Wedge
2	Red Silicone Seals	1	Optical Bench Lock
1	Inlet Barb	2	PTFE Capillary Cover

Parts Kit includes

Quantity	Description	Quantity	Description
2	Inlet Seal Fitting	1	Optical Bench
7	Red Silicone Seals	4	Retainer Wedge
2	Inlet Barb	1	Optical Bench Lock
1	Polyurethane Tubing, 4 meters	4	PTFE Capillary Cover
2	Outlet Barb		

Assembly materials include

Quantity	Description	Quantity	Description
1	Alignment Tool	3	Applicator Swab
1	White Ceramic Square	1	Tube of Vacuum Grease
1	PTFECapillary Guide Tubing, 1 meter	1	Inlet Seal Installation Tool
1	Sand Paper		

Product Information

Catalog Number	Product Description
148-6072	**Inlet Seal Fitting,** 5
148-6073	**Inlet Barbed Fitting,** 5
148-6074	**Outlet Barbed Fitting,** 5
148-6075	**Red Silicone Seal,** 10
148-6077	**Tubing, Polyurethane for capillary coolant,** 12 feet (4 meters)
148-6078	**Optical Bench (1 each) and Retainer Wedges (3 each)**
148-6079	**Optical Bench Lock,** 1
148-6080	**Cartridge Cover,** 1
148-6081	**Tubing, PTFE for capillary guide,** 3 feet (1 meter)
148-6082	**Ceramic Square for cleaving Capillary**
148-6083	**Installation Tool for Inlet Seal Fitting**
148-6084	**Alignment Tool**

To order or for Technical Service, call 1-800-4BIORAD, 1-800-424-6723.

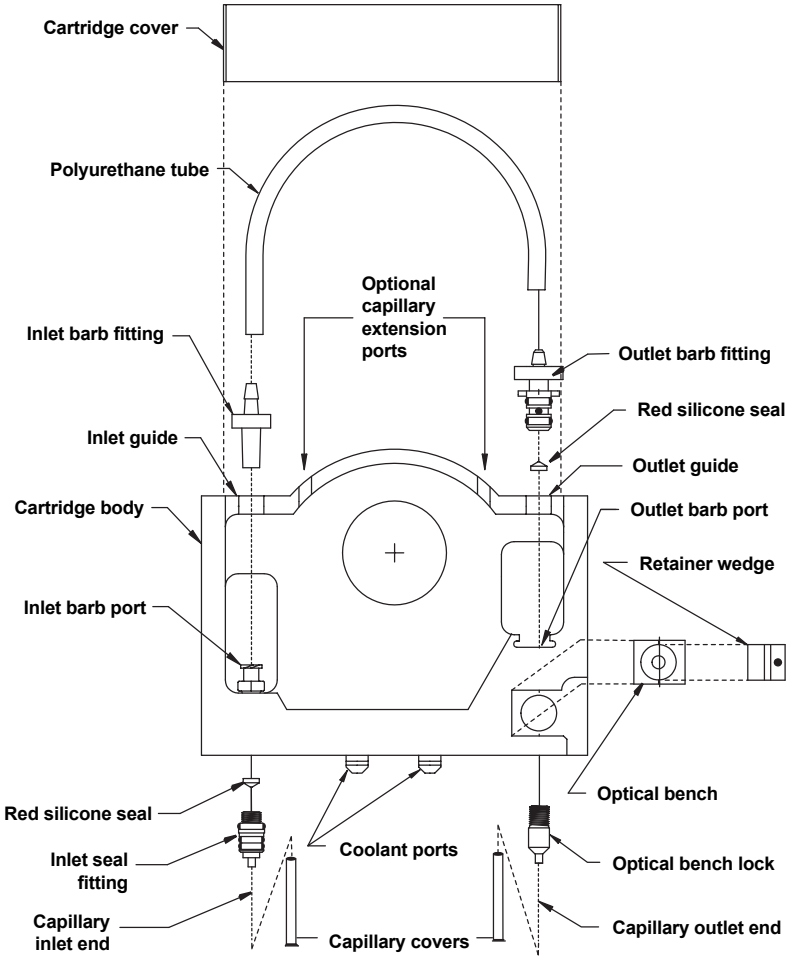

Fig. 1. BioFocus cartridge assembly diagram.

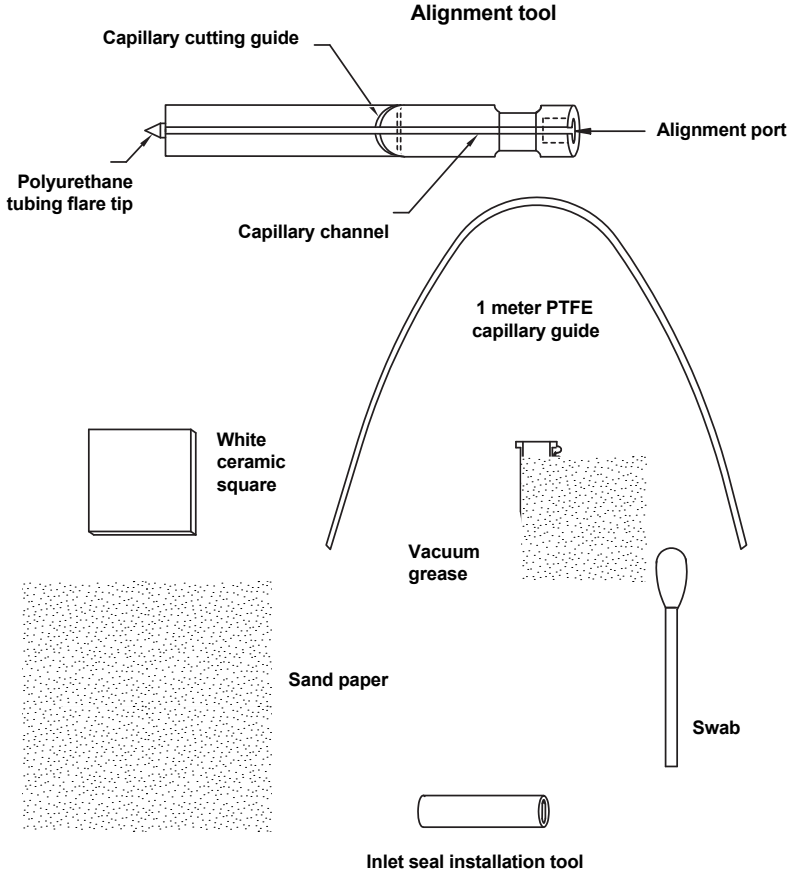

Fig. 2. Cartridge assembly tools.

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