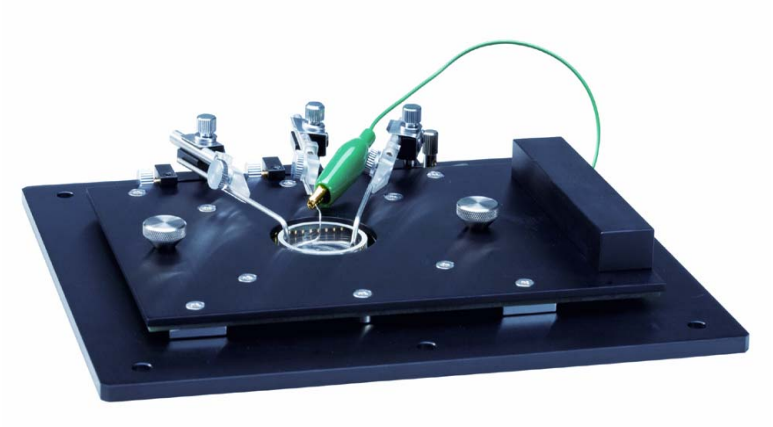
MED64 The most sensitive microelectrode array system for *in vitro* extracellular electrophysiology

Product Manual

Perfusion Pipe Holder Kit

P/N: MED-KPK02(TU)



ALPHA MED SCIENTIFIC

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Contents

1. Perfusion Pipe Holder Kit (MED-KPK02)

The perfusion Pipe Holder Kit (MED-KPK02) is an accessory for the MED64 System. It is installed onto the MED Connector (or MED Heated Connector) and provides the capability to perfuse solution in the Probe chamber combined with an peristaltic pump. The Perfusion Pipe Holder Kit **fits ONLY the MED Probe with 5 mm chamber height (MED-PXXX5).**

The Perfusion Pipe Holder Kit (MED-KPK02) consists of following items (Figure 1):

1. Perfusion Pipe Holder (MED-KPH02): 3 units
2. Inlet Pipe (MED-KPS02, hole at the bottom): 1 unit
3. Outlet Pipe (MED-KPD02, hole at the side): 1 unit
4. Ref Electrode (MED-KPR02, Platinum wire): 1 unit
5. Wrench: 1 unit

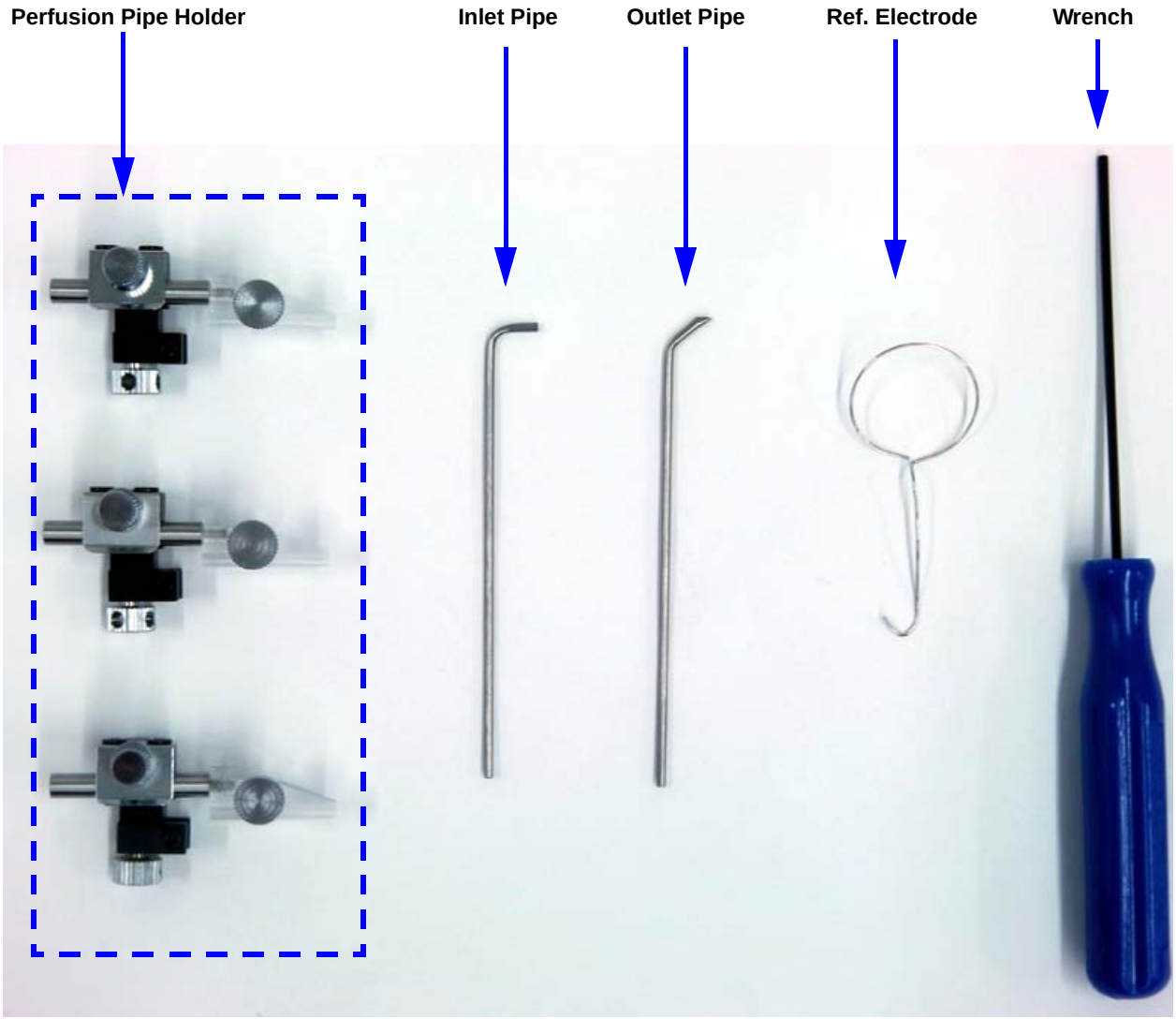


Figure 1. Perfusion Pipe Holder Kit.

1-1. Perfusion Pipe Holder

The Perfusion Pipe Holder holds the Inlet/Outlet Pipe or Ref Electrode. It consists of 1) Holder unit to hold pipe or ref electrode and 2) Base unit to be installed to the MED (Heated) Connector. It has 3 screws with the following functions:

Top screw: To adjust the height and distance for the Pipe/Ref Electrode (Figure 3).
Middle screw: To hold Pipe/Ref Electrode (Figure 4).
Bottom screw: To change the location (right or left) and height of the pipe holder (Figure 5).

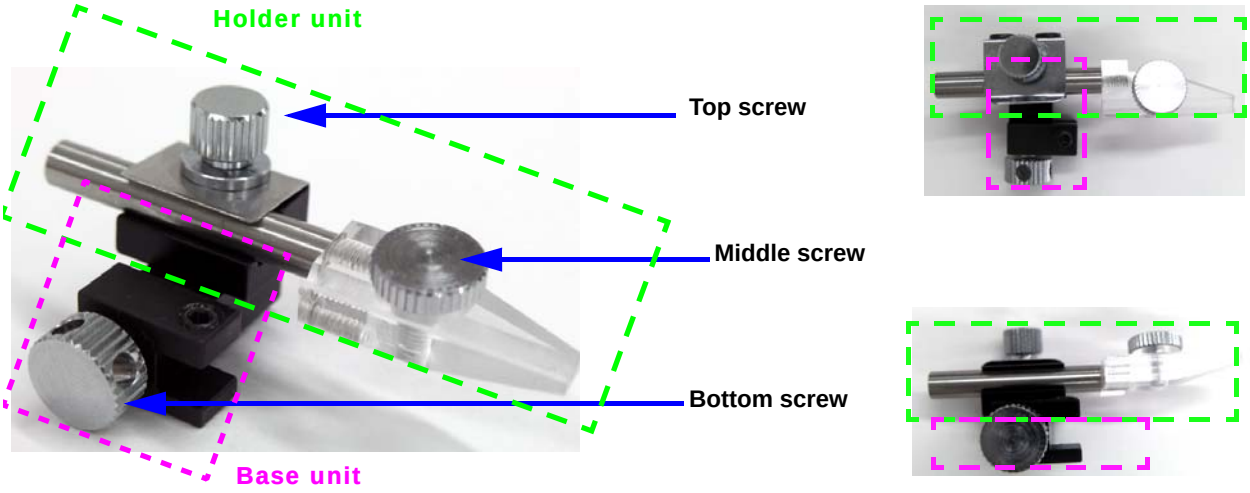


Figure 2. Perfusion Pipe Holder. Top view (top-right) and side view (bottom-right).

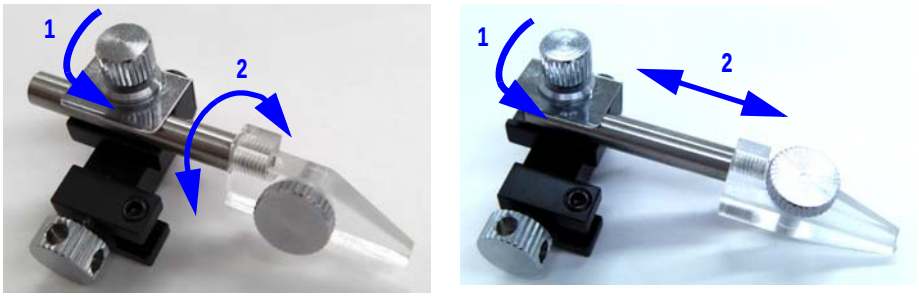


Figure 3. Functions for the Top screw. Loosening the screw and rotating the bar will change a height for the pipe/ Ref. Electrode (left). Loosing the screw and sliding the bar will change the distance of the pipe/ Ref. Electrode to the MED Probe chamber (right).

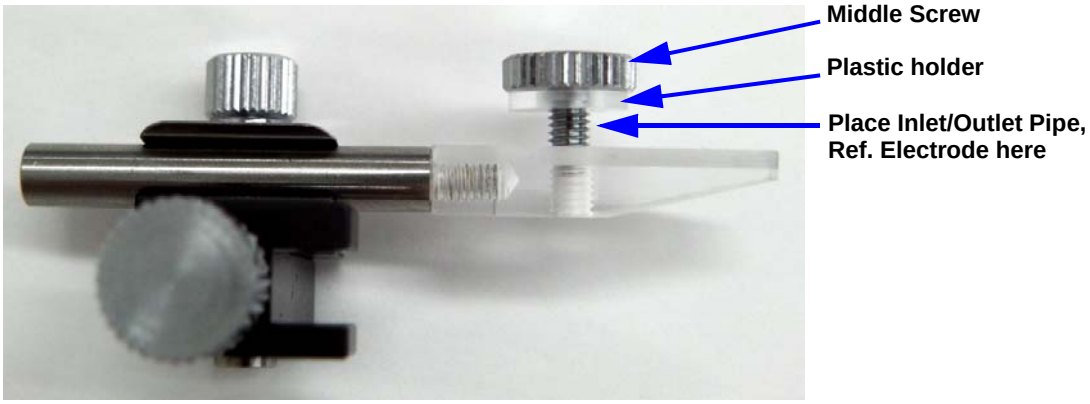


Figure 4. Function for the Middle screw. It holds the pipe/Ref. Electrode or let them go.

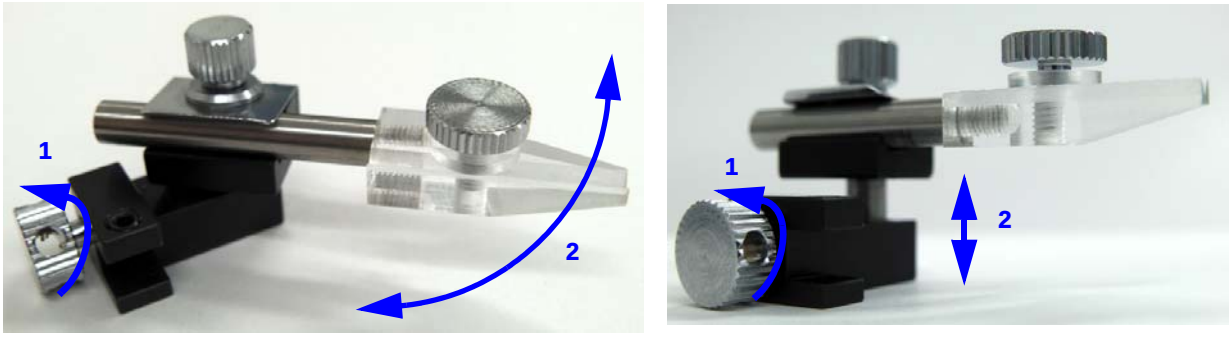


Figure 5. Function for the bottom screw. Loosening this screw will move the holder unit right and left (left) or/and change its height (right).

2. Installating the Perfusion Pipe Holder Kit

Install the Perfusion Pipe Holder Kit with following procedure:

1. Install the Ref. Electrode and Inlet/Outlet pipes to Perfusion Pipe Holders.
2. Install the pipe holder holding the Ref. Electrode onto the MED (Heated) Connector.
3. Install the pipe holders hodling Inlet/Outlet Pipes to the MED Connector.

2-1. Installing the Ref. Electrode to the Perfusion Pipe Holder

1. Loosen the middle screw and hook the u-shaped ede of the Ref. Electrode to the middle screw. Place the Ref Electrode under the plastic holder to achieve stability (Figure 6).

2. Tighten the screw until the Ref. Electrode is fixed and unmoved.

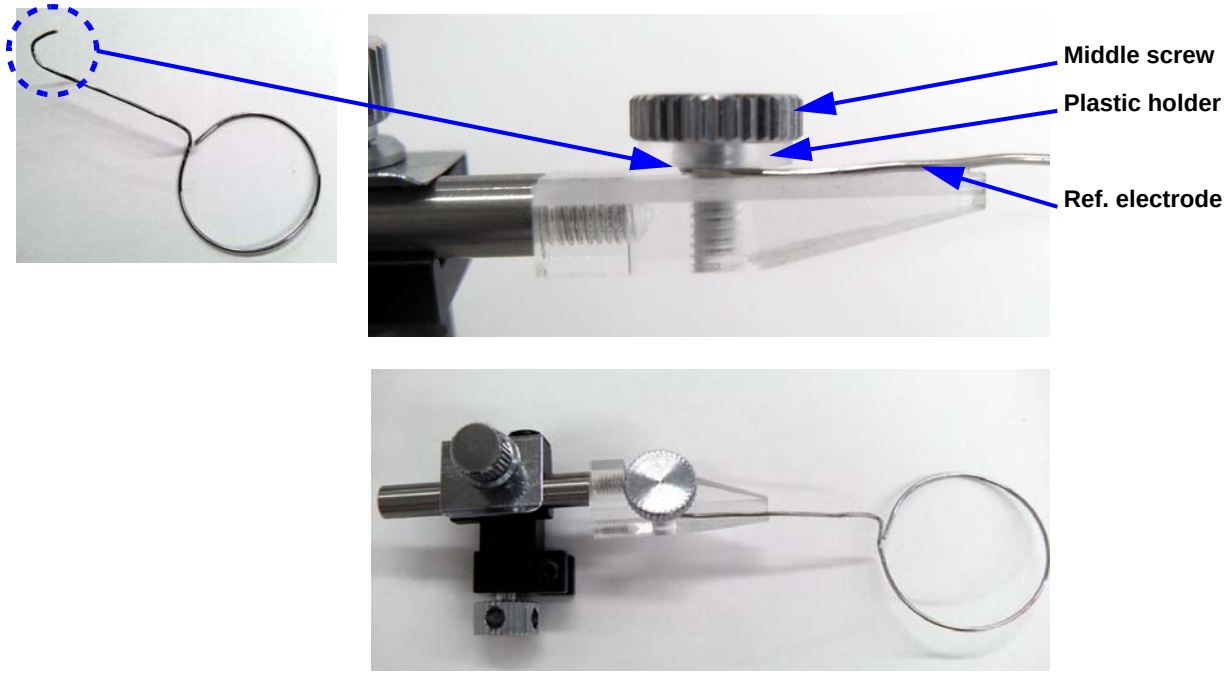


Figure 6. Installing the Ref. Electrode to the Perfusion Pipe Holder.

2-2. Installing the Inlet/Outlet Pipes to the Perfusion Pipe Holders

Please note that the tip shape for the Inlet and Outlet Pipes are different. The Inlet pipe is designed so that it has a hole in the bottom while the Outlet Pipe is designed so that it has a hole at the side.

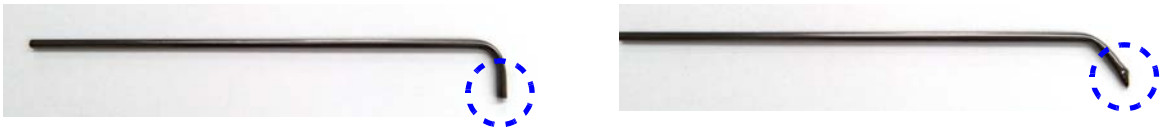


Figure 7. The Inlet (left) and Outlet (right) Pipe. The Inlet Pipe is designed for having a hole in the bottom while the Outlet Pipe is designed for having a hole at the side.

1. Connect the Inlet/Outlet Pipes to the perfusion tubing.
   - *When the Perfusion Pipe Holder Kit with Tube (MED-KPK01TU) is purchased, skip this process and proceed to the step 2 on the page 6.*

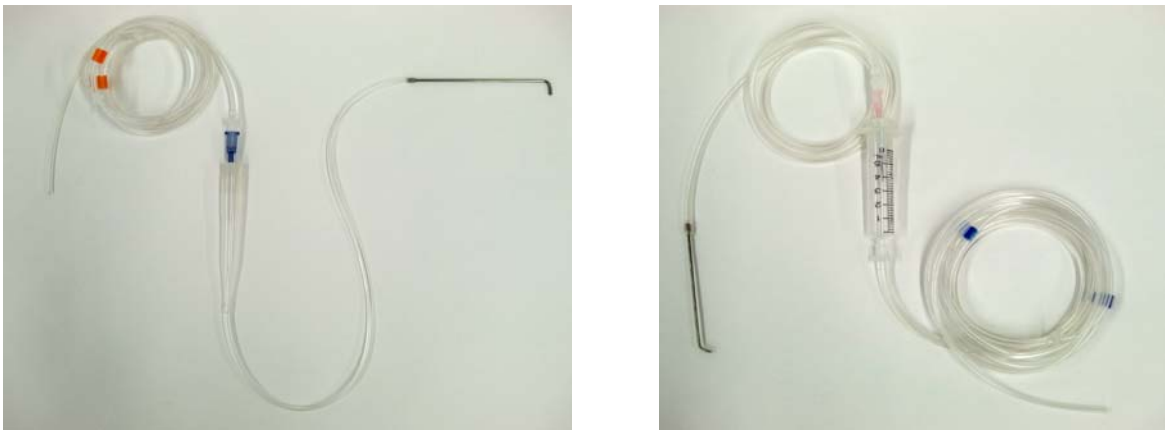


Figure 8. Inlet Pipe (left) and Outlet Pipe (right) connected to perfusion tubing.

NOTE:

It is recommended to use a tube with larger internal dimension for the outlet side (around twice as large as the inlet pipe) to make solution flow smoothly and prevent solution from overflowing over the MED Probe. Connecting the large-diameter tube directly to the Outlet Pipe can not make firm connection. Thus, use a short small-diameter tube to connect the large-diameter tube to the outlet pipe firmly (Figure 9).

Figure 9. Connecting the outlet tube to the Outlet Pipe.

2. Loosen the middle screw and insert a pipe under the plastic holder (Figure 10). Tighten the middle screw so that the pipe is not moved. Figure 11 shows the Inlet and Outlet Pipes installed to the Perfusion Pipe Holders.

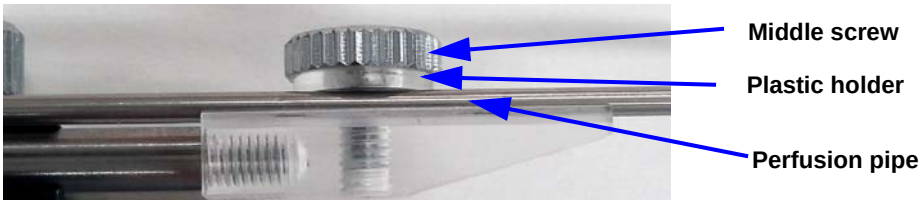


Figure 10. Location of Inlet (or Outlet) pipe in the Perfusion Pipe Holder.

Figure 11. Inlet (left) and Outlet (right) Pipes installed to the Perfusion Pipe Holders.

2-3. Installing the Perfusion Pipe Holders to the MED (Heated) Connector

Recommended layout

The Perfusion Pipe Holders which hold Inlet/Outlet Pipes and Ref. Electrode will be installed to the top unit of the MED (Heated) Connector. **It is very important to create solution flow over the sample (electrodes).** To do this, **install the pipe holders so that tips for the Inlet and Outlet pipes are located in a line across electrodes** (Figure 12). Figure 13 and 14 shows the layouts recommended to achieve this.

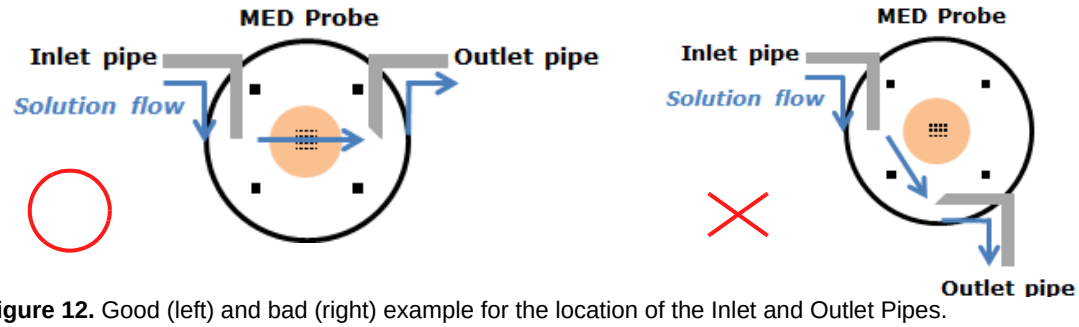


Figure 12. Good (left) and bad (right) example for the location of the Inlet and Outlet Pipes.

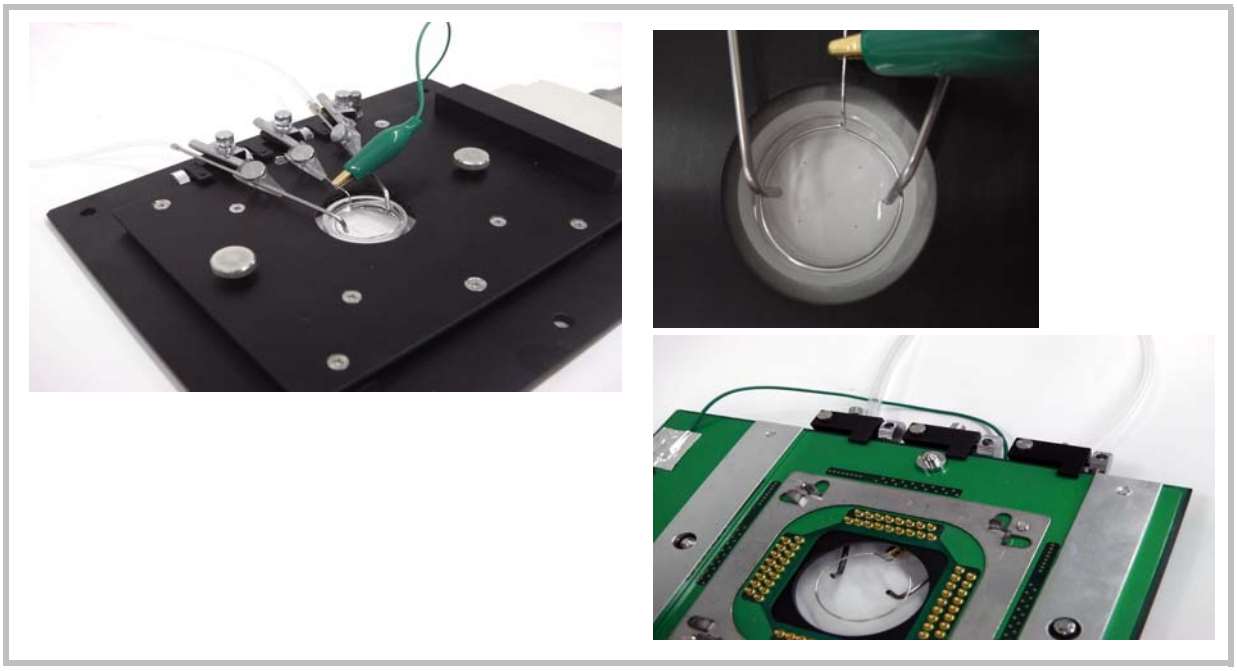


Figure 13. Recommended layout-1 (for MED Connector, MED-C03 only).

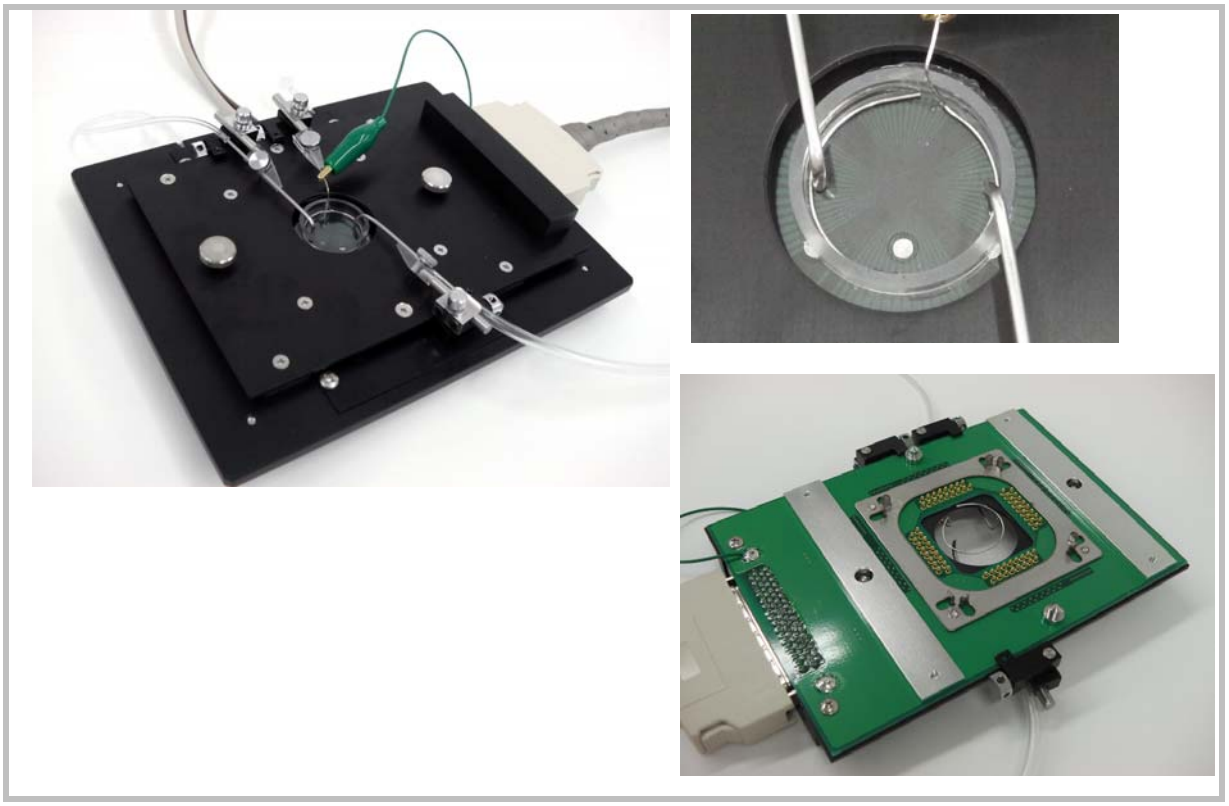


Figure 14. Recommended layout 2.

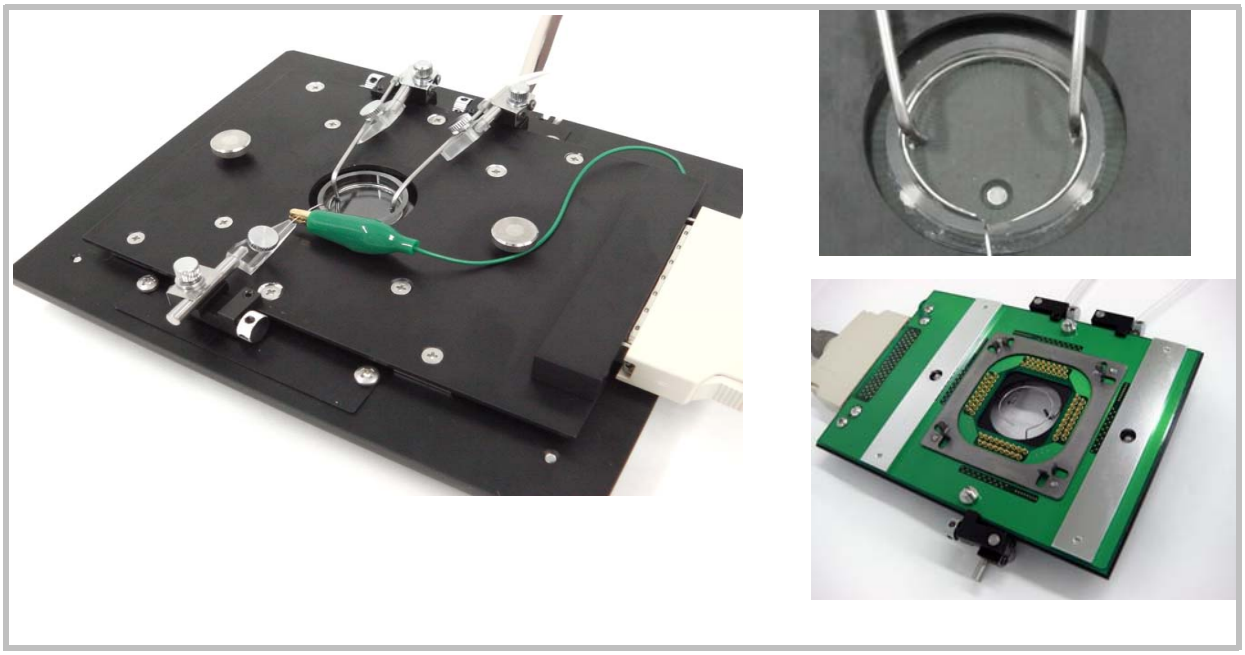


Figure 15. Recommended layout 3.

Installing the Perfusion Pipe Holders to the MED (Heated) Connector

CAUTION:

Do NOT touch the contact pins in the MED (Heated) Connector with your bare hands or finger tips or moisten the pins, which may cause rust and adversely affect the electrical conduction.

1. Loosen the thumb screws on the MED (Heated) Connector and remove the top unit from the bottom unit (Figure 16).

2. Slide the groove of a Pipe Holder holding the Ref. Electrode on to the top unit of the MED (Heated) Connector (Figure 17).

   NOTE:

   Installing the Ref. Electrode first will make following installations easier.

3. Tighten the hexagon head socket with the Wrench until the Perfusion Pipe Holder is fixed firmly onto the MED (Heated) Connector(Figure 18).

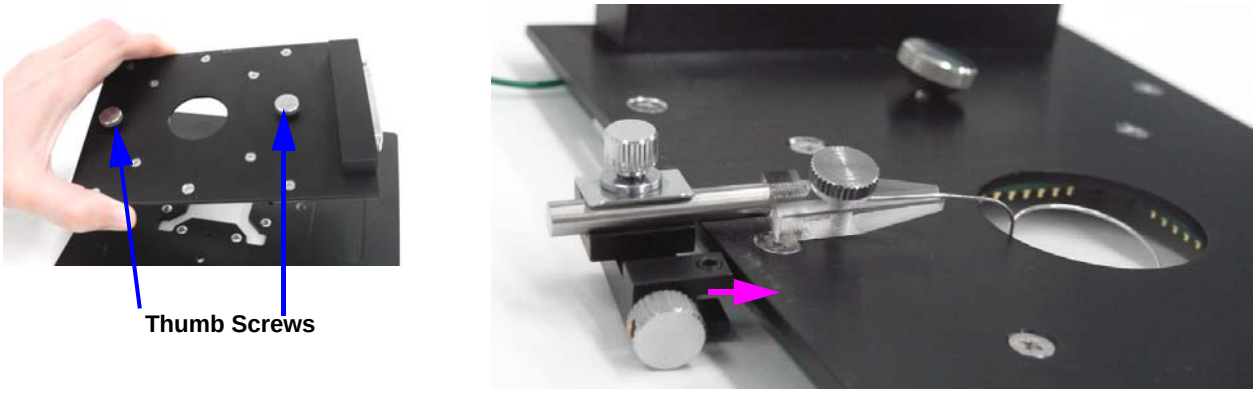


Figure 16. Removing the top unit from the bottom unit.
Figure 17. Installing the Pipe Holder to the top unit of the MED Connector.

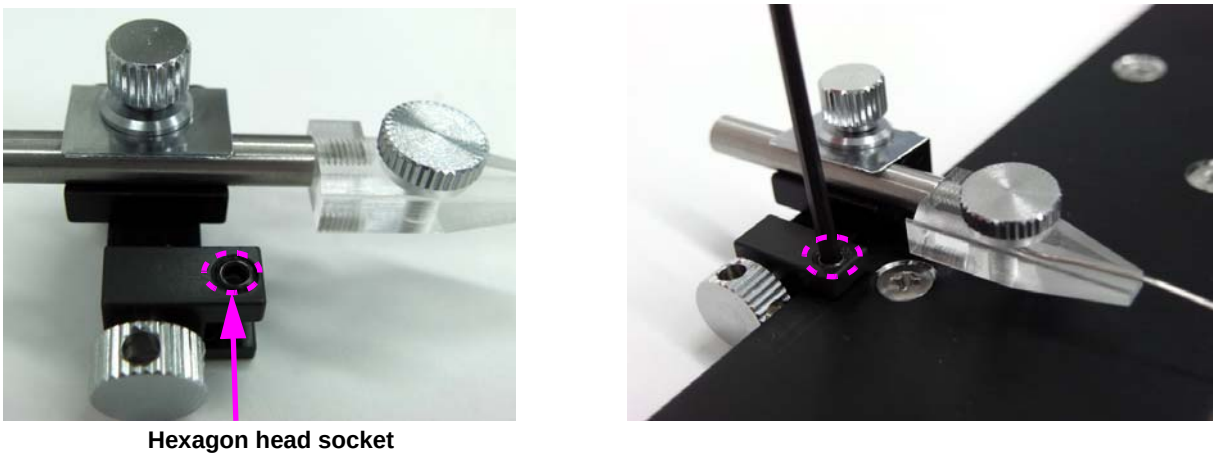
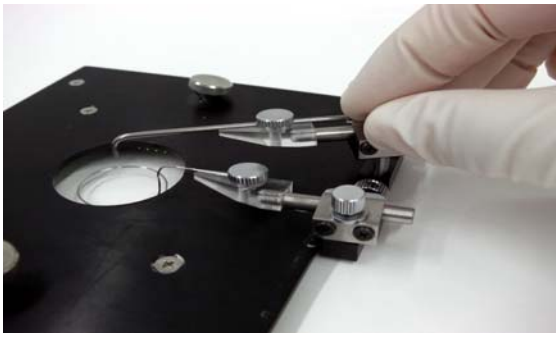


Figure 18. Fixing the Pipe Holder to the MED Connector.

4. Install another pipe holders (holding the Inlet/Outlet Pipes) to the top unit of the MED (Heated) Connector with same procedures (Figure 19). Adjust the location or/and height for the Inlet/Outlet Pipes' tips by loosening and tightening the bottom screw (Use the Wrench if necessary, Figure 20).

   Select the location for the Perfusion Pipe Holder NOT being on a screw in the bottom unit of MED Connector.



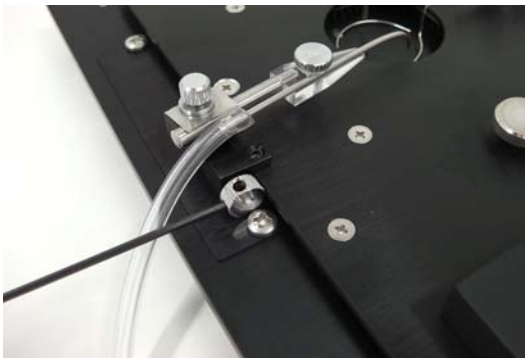


Figure 19. (Left) Installing the Perfusion Pipe Holder holding the Inlet Pipe.
Figure 20. (Right) Fixing the location for the Outlet Pipe using the Wrench. PIpe holder should NOT be on the top of screws in the bottom unit.

5. Mount the MED Probe onto the bottom unit for the MED (Heated) Connector and replace the top unit on the bottom unit.

   Note:

   It is recommended to use an old MED Probe for initial setup.

6. Adjust the location and height for the pipe tip. In order to create smooth solution flow without interference of perfusion noise, it is recommended to locate tips of Inlet/Outlet Pipes as followings:

   Inlet Pipe: Slightly higher than bottom of the MED Probe
   Outlet Pipe: Slightly higher than the tip of Inlet Pipe.

   In order to position the Inlet/Outlet Pipes such taht the positions are low enough for good perfusion, it is necessary to rotate the Pipe Holder, as demonstrated in Figure 21. Lower the pipe tip by rotating the pipe holder to hold the pipe at lower position. This can be made with following procedure:

   1) Loosen the top screw and rotate the pipe holder anticlockwise (Figure 21, A & B).
   2) Change the location of pipes' tip to the bottom (by loosing the Middle screw and rotating the inlet pipe clockwise) (Figure 20, B & C).
   3) Fix the location by tightening both screws.

7. Figure 22. shows Perfusion Pipe Holder Kit with Inlet ant Outlet Pipes installed appropriately.

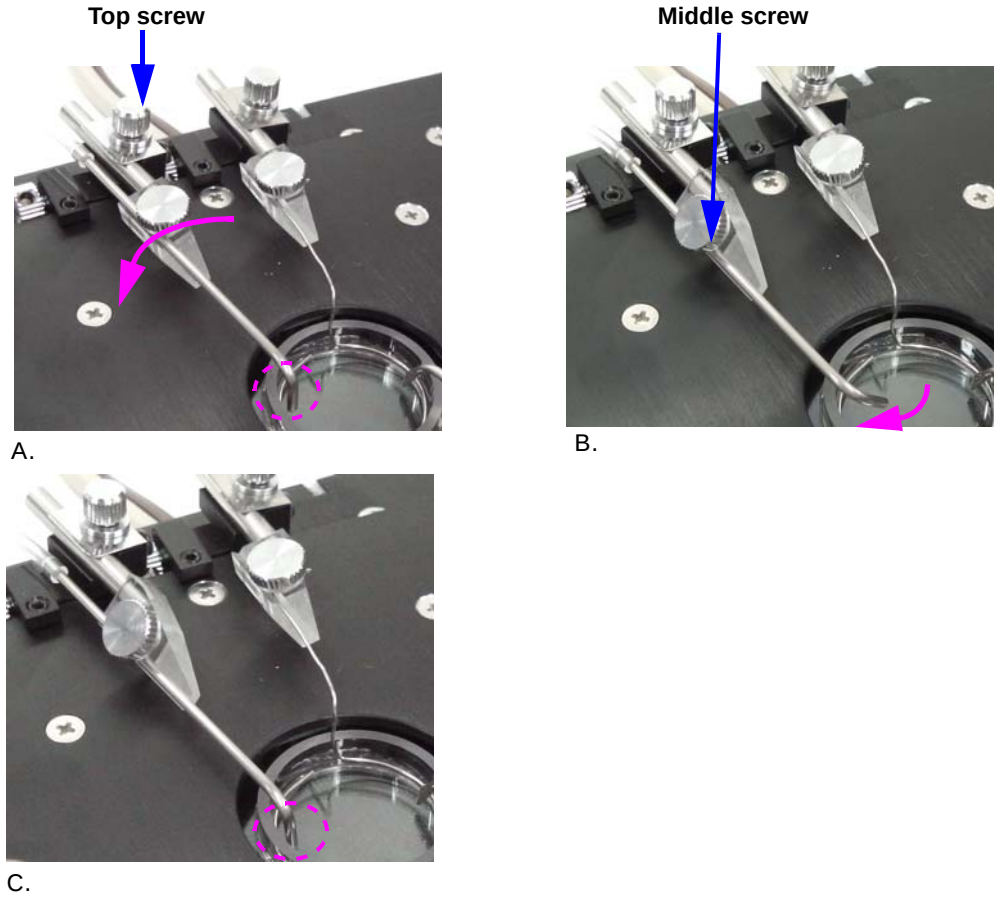


Figure 21. The original position for the Inlet/Outlet Pipes' tips are not low enough to create stable solution flow. (A). Let the pipe holder to hold the pipe at lower position using the top screw (A & B), and then change the position for the tip using the Middle screw. (B & C)

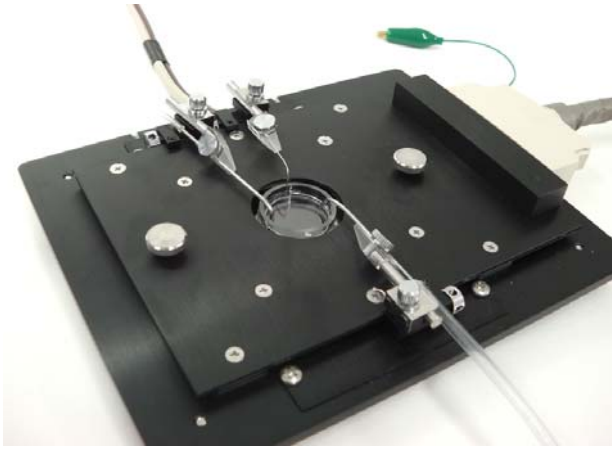


Figure 22. Perfusion Pipe Holder Kit installed to the MED Heated Connector.

8. Ground the Ref. Electrode to the top unit of the MED (Heated) Connector (Figure 23). **This grounding is necessary to let the Ref. Electrode to work as a reference electrode.** If the Ref. Electrode is not grounded, large noise will be present in the recordings.

The Ref. Electrode (platinum wire)

MED Probe has 4 reference electrodes embedded as well as 64 recording electrodes. However, addition of this Ref. Electrode works for:

1. Supress dragging of stimulus artifacts.
2. Remove perfusion noise.
3. Decreasing the total impedance in a MED Probe. (Making the baseline noise even lower)

Make sure for the Ref. Electrode sits on the bottom of MED Probe covered by solution, and doesn't move. If perfusion noise is still observed with this grounding, ground the Ref. Electrode (platinum wire) to the inlet pipe (Figure 23, right).

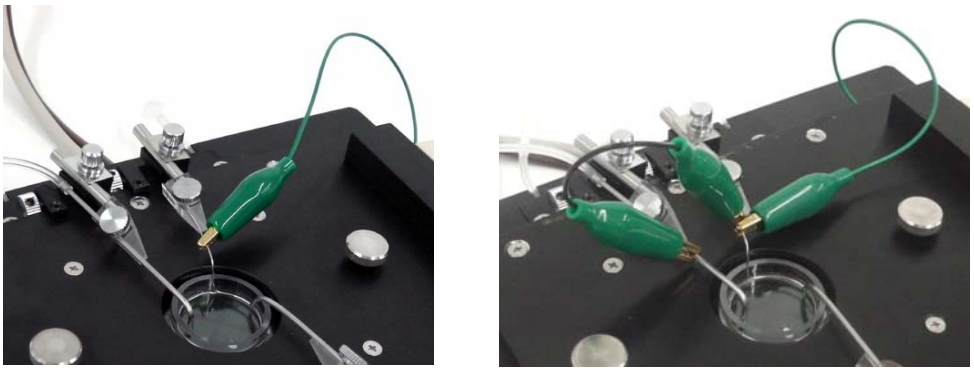


Figure 23. Grounding the Ref. Electrode to the MED Heated Connector.

3. Perfusion Pipe Holder Kit with Tubing

3-1. Perfusion Pipe Holder Kit with Tubing

The Perfusion Pipe Holder Kit with Tubing (MED-KPK02TU) comes with perfusion tubes ready-to-go. This section will guide you how to install the tubes to your peristaltic pump.

The Perfusion Pipe Holder Kit with Tubing is compatible with Minipuls 3 manufactured by Gilson Inc. When another type of peristaltic pump is used, replace the tubes with the correct size for your peristaltic pump.

The Figure 24-26 shows the Inlet/Outlet Pipe and tubing and accompanying accessories.

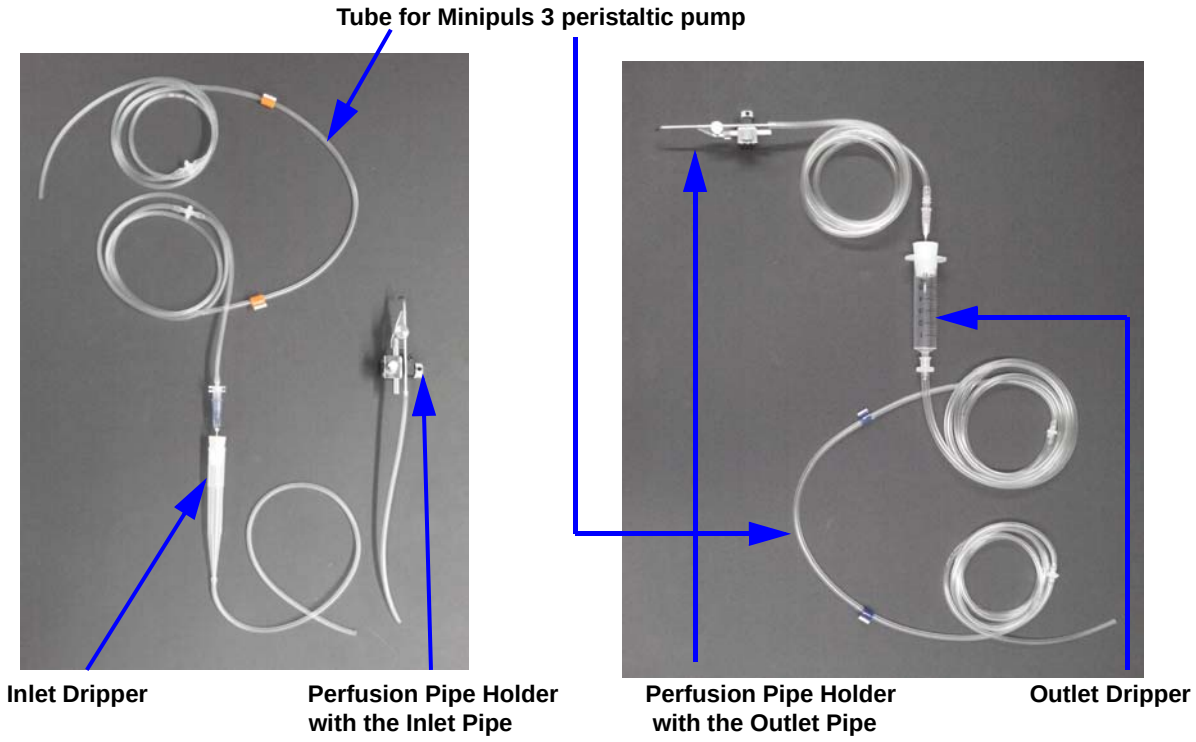


Figure 24. (Left) Perfusion Pipe Holder with Inlet Pipe and tubing.
Figure 25. (Right) Perfusion Pipe Holder with Outlet Pipe and tubing.

Figure 26. Accessories for the MED-KPK02TU.

Install the tubes as seen in the Figure 27 and 28.

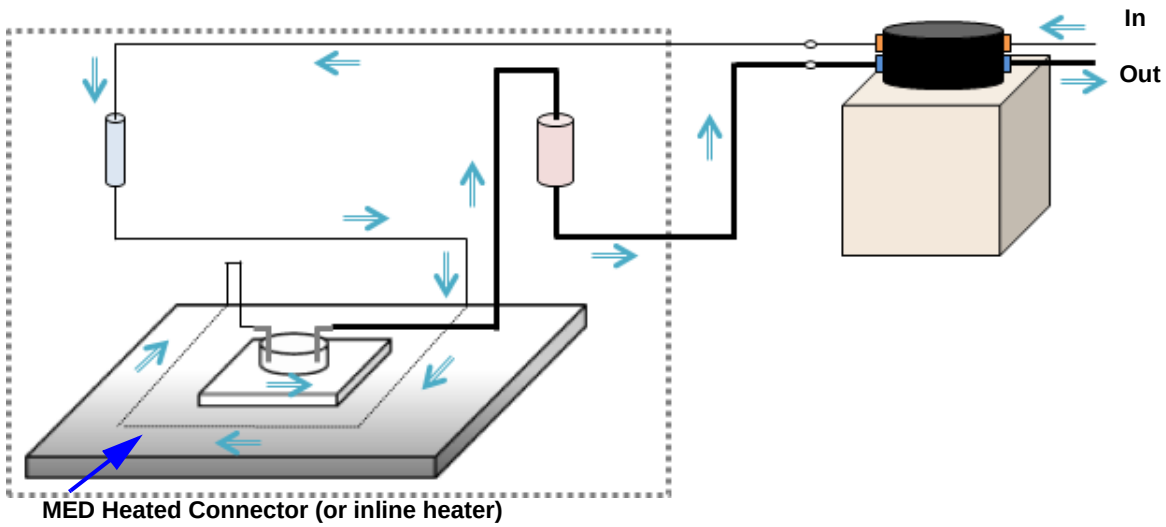


Figure 27. Installation of the tubes to the peristaltic pump.

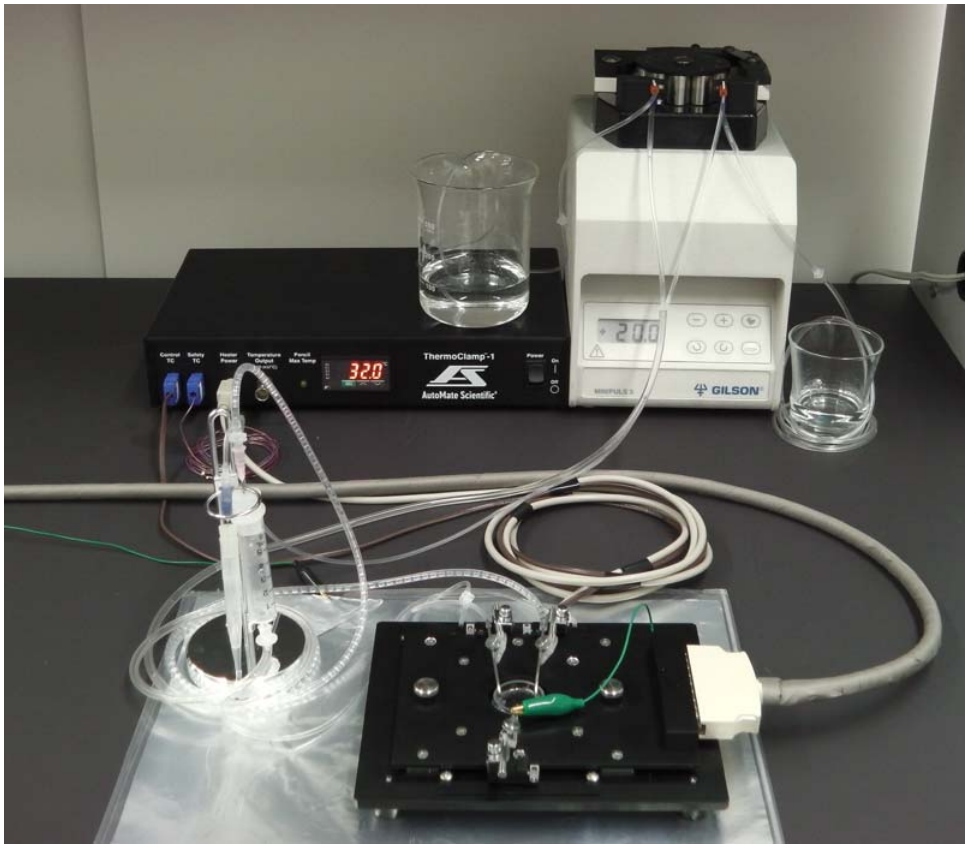


Figure 28. Perfusion Pipe Holder Kit with Tubing installed to the Minipuls 3 peristaltic pump.

3-2. Installing the perfusion tubing to the Minipuls 3 peristaltic pump

CAUTION:

Please read instruction for the Minipuls 3 before the installation.

1. Open the Locking key and Compression cam (Figure 29).

2. Hook the perfusion tube to the rollers in the Minipuls 3 (Figure 30).
   Make sure that tubes are installed horizontally and with same orientations so that solution won't flow backwards (Figure 31, 32).

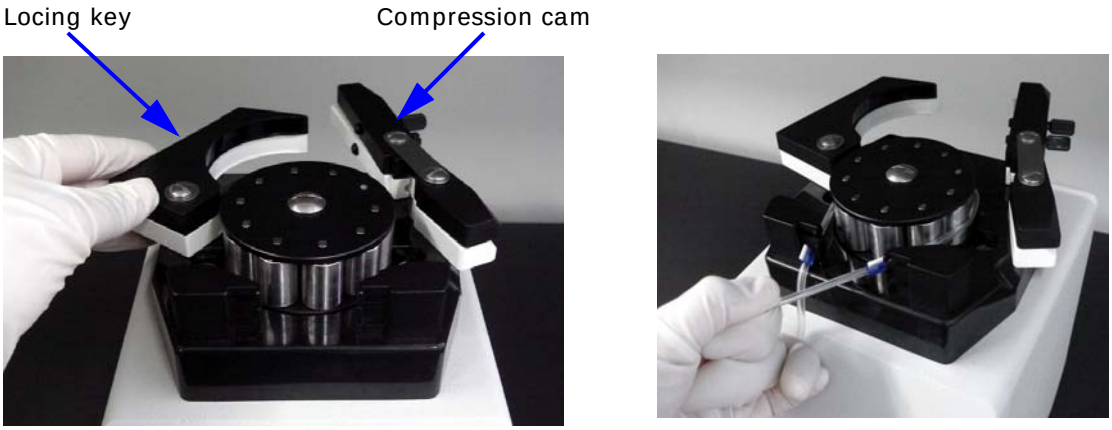


Figure 29. (Left) Opening the Locking key and Compression cam.
Figure 30. (Right) Installing the inlet tube to the Minipuls 3.

Figure 31. Perfusion tube installed in the Minipuls 3 . Make sure both tubes have same orientations. The right shows the back-view.

Figure 32. Bad examples for the tubes installed.

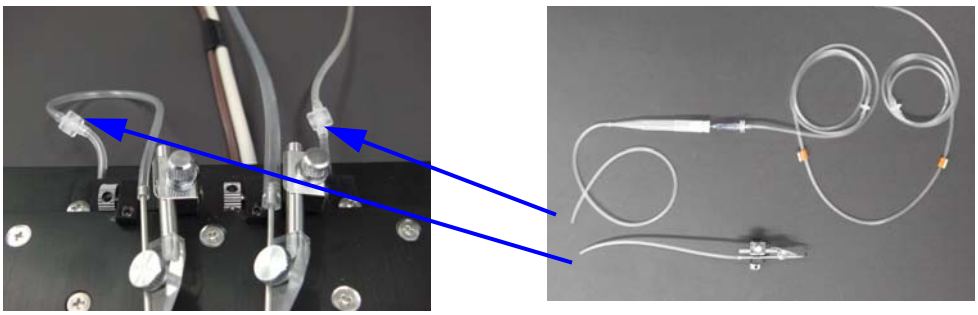
3. Relocate the Compression cam and Locking key (Figure 33).
   Make sure that the inlet/outlet tubes sit UNDER the Compression cam.

4. Adjust the pressure using the Adjustment screw in the back (Figure 34).

Figure 33. (Left) Placing back the Compression cam and Locking Key.
Figure 34. (Right) Adjusting the pressure using the Adjustment screw in the back.

3-3. Installing the perfusion tubing to the “perfusion tube port” in the MED Heated Connector

Connect the inlet tube to the “perfusion tube port” in the MED Heated Connector (or your in-line heater) so that solution will be warmed up before sent to the MED Probe chamber.



Inlet PIpe

Figure 35. Connecting the inlet tube to the MED Heated Connector.

3-4. Installing the Inlet/Outlet drippers

The Inlet/Outlet drippers function to remove electrical noise by breaking the continuity of the recording solution.

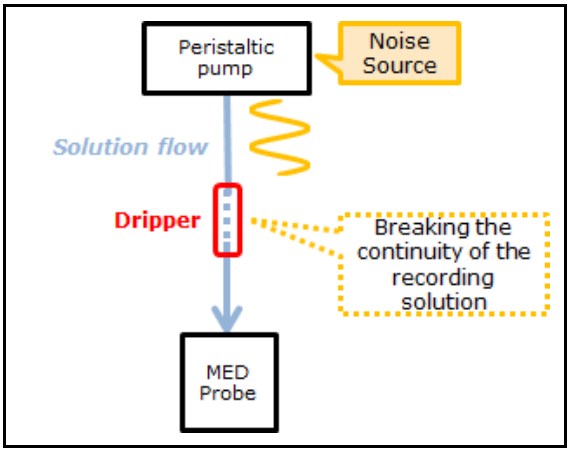


Figure 36. Inlet and Outlet drippers stranded at the dripper stand (left), and how they work to remove noises (right).

Disconnect the syringe and the rubber cap to install the drippers in the dripper stand (Figure 37).

Figure 37. Installing the outlet dripper to the dripper stand.

Figure 38 shows an example for how the dripper is installed in the configuration with microscope.

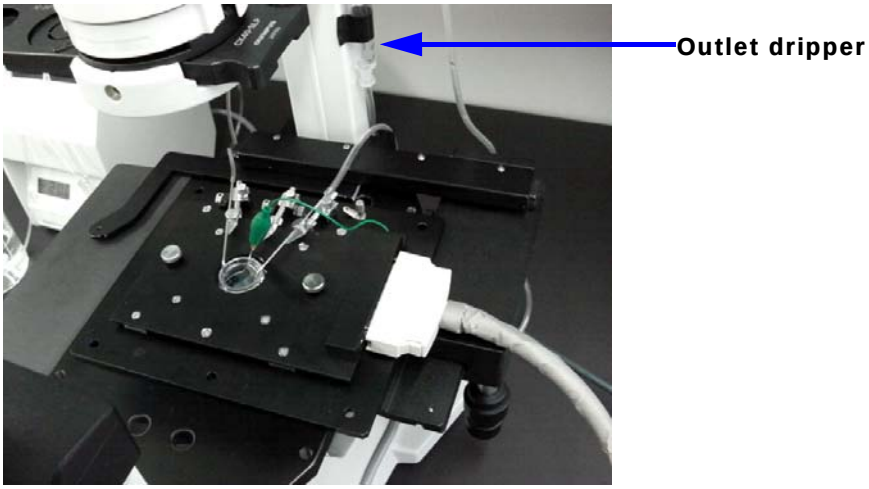


Figure 38. Perfusion Pipe Holder Kit with Tubing and MED Connector installed on a microscope stage.

4. Instructions for use and maintenance

4-1. Perfusion Pipe Holder Kit

Important tips for your perfusion

- For acquisitions with low-noise, the Ref. Electrodes must:
    - NOT touch the Inlet/Outlet Pipes.
    - Sit in the bottom of the MED Probe and NOT move.
    - be completely covered by the solution.
- Be careful not to flow solution over the MED (Heated) Connector. Particularly, pay extra attention when starting perfusion. (Solution could become higher for a moment before the suction starts.)
- Be careful not to scratch the bottom of MED Probe with the Inlet/Outlet Pipes, which might damage the surface of a MED Probe (insulation layer, electrodes, and leads). On the other hand, make the Inlet Pipe as low as the tip is in the solution. (Dropping could fluctuate solution in the MED Probe chamber, which introduce noise.)

Cleaning

- CLEAN the Inlet/Outlet Pipes and Ref. Electrode with kimwipe soaked with 70% ethanol.
- Soak the Inlet/Outlet Pipes to acetone or ethanol sometimes to clean inside of the pipes.

Sterilization

- Soak pipes and ref. electrode in ethanol to sterilize.
- For the Perfusion Pipe Holder, clean them with a kimwipe soaked with ethanol.
  Avoid spraying with ethanol, as too much ethanol may cause the acrylic of the Perfusion Pipe Holder to crack.

Others

- Do NOT bend the Inlet/Outlet Pipes.
- Do NOT tighten the screws excessively to avoid damaging them.
- Avoid mechanical shock.

4-2. Perfusion Tubing

- Clean the tube with distilled/Milli-Q water for at least 10 minutes **after every experiment.**
- Release the perfusion tubing from the peristaltic pump when not used. Failing to do this will stretch and wear out the tubes quickly.
- Replace the tubes/drippers with new ones often. Using old tubes prevents solution from flowing smoothly and causes perfusion noise. The following are recommendations for the replacement when perfusion is used daily:

    - Tubes for peristaltic pump: 2-3 weeks
    - Inlet/outlet drippers: 1 month
    - Other tubes: 2-3 months

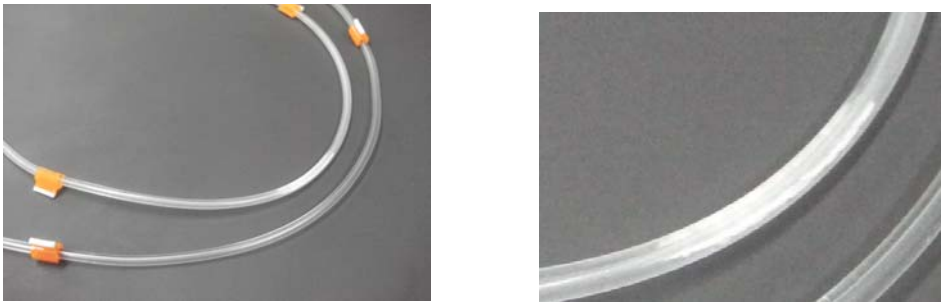


Figure 39. Examples for the tubes to be replaced due to cracks.

Note:

Lure fitting can be crushed easily when connected to a tube by hands. It is recommended to make the tubing wider using a pipet-tip before connecting a lure fitting (Figure 40).

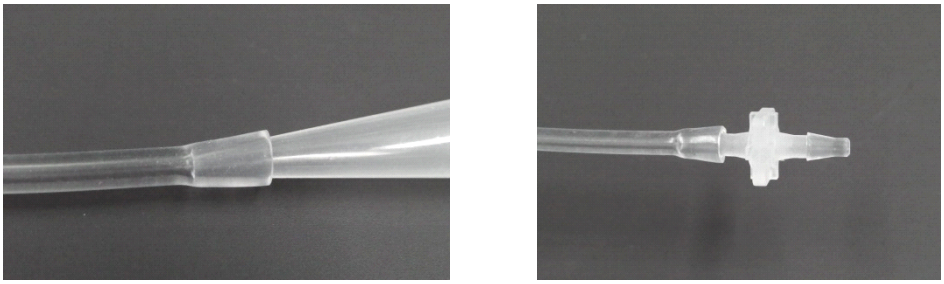


Figure 40. Making a tube-tip wider first (left), and then connect it to a lure fitting (right).

5. Specifications

5.1. Perfusion Pipe Holder Kit

Perfusion Pipe Holder	
Material	Stainless, Acrylic
Perfusion Pipe	
Material	Stainless
Size	OD: φ1.48 mm ID: φ1.2 mm Length: 55 mm

5-2. Inlet tubing

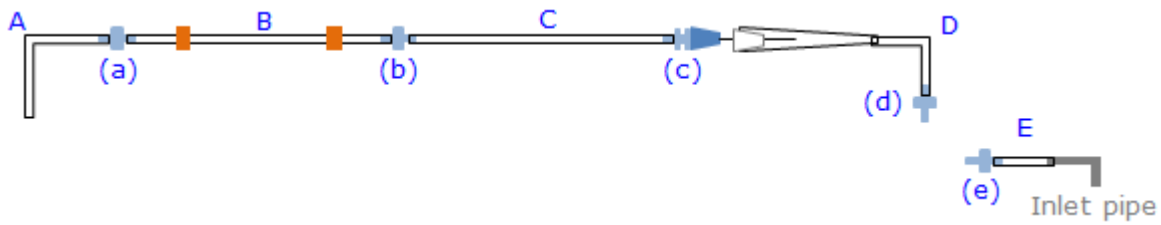


Figure 41. Inlet pipe and tubing.

	I.D/O.D (mm)	Length (cm)	Manufactures/ Parts No.
A,C	0.79/2.38	50	ACF00001 (E-3603, Tygon S3™)
B	0.89 (I.D) (For Peristaltic pump)	-	F117937 (Gilson®)
D	0.79/2.38	30	ACF00001 (E-3603, Tygon S3™)
E	0.79/2.38	11	ACF00001 (E-3603, Tygon S3™)
(a), (b), (d), (e)	Lure fitting, for 2.5mm ID		
(c)	Lure fitting , for 1.5mm ID,		

Table 1. Specifications for the Inlet tubing

5-3. Outlet tubing

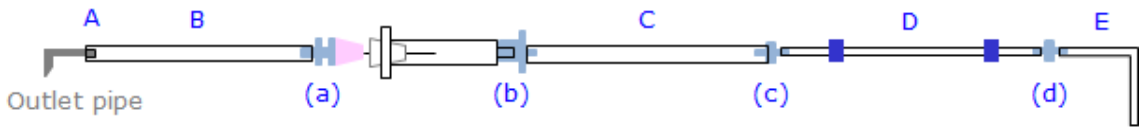


Figure 42. Outlet pipe and tubing.

	I.D/O.D (mm)	Length (cm)	Manufactures / Parts No.
A	0.79/2.38	0.5	ACF00001 (E-3603, Tygon S3™)
B	2.38/3.97	90	ACF00004 (E-3603, Tygon S3™)
C		85	ACF00003 (E-3603, Tygon S3™)
D	1.65 (I.D) (For Peristaltic tube)	-	FF17943 (Gilson®)
E	1.59/3.18	100	ACF00002 (E-3603, Tygon S3™)
(a)	Lure fitting, for 2.5mm ID		
(b)	Lure fitting, for 1.5mm ID		
(c), (d)	Lure fitting, for 1.5 mm ID		

Table 2. Specifications for the outlet tubing.

5-4. Inlet/outlet drippers

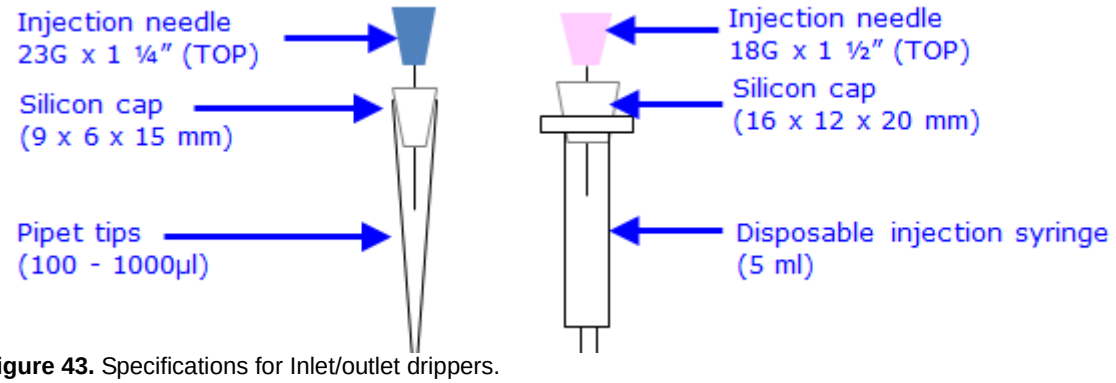


Figure 43. Specifications for Inlet/outlet drippers.

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