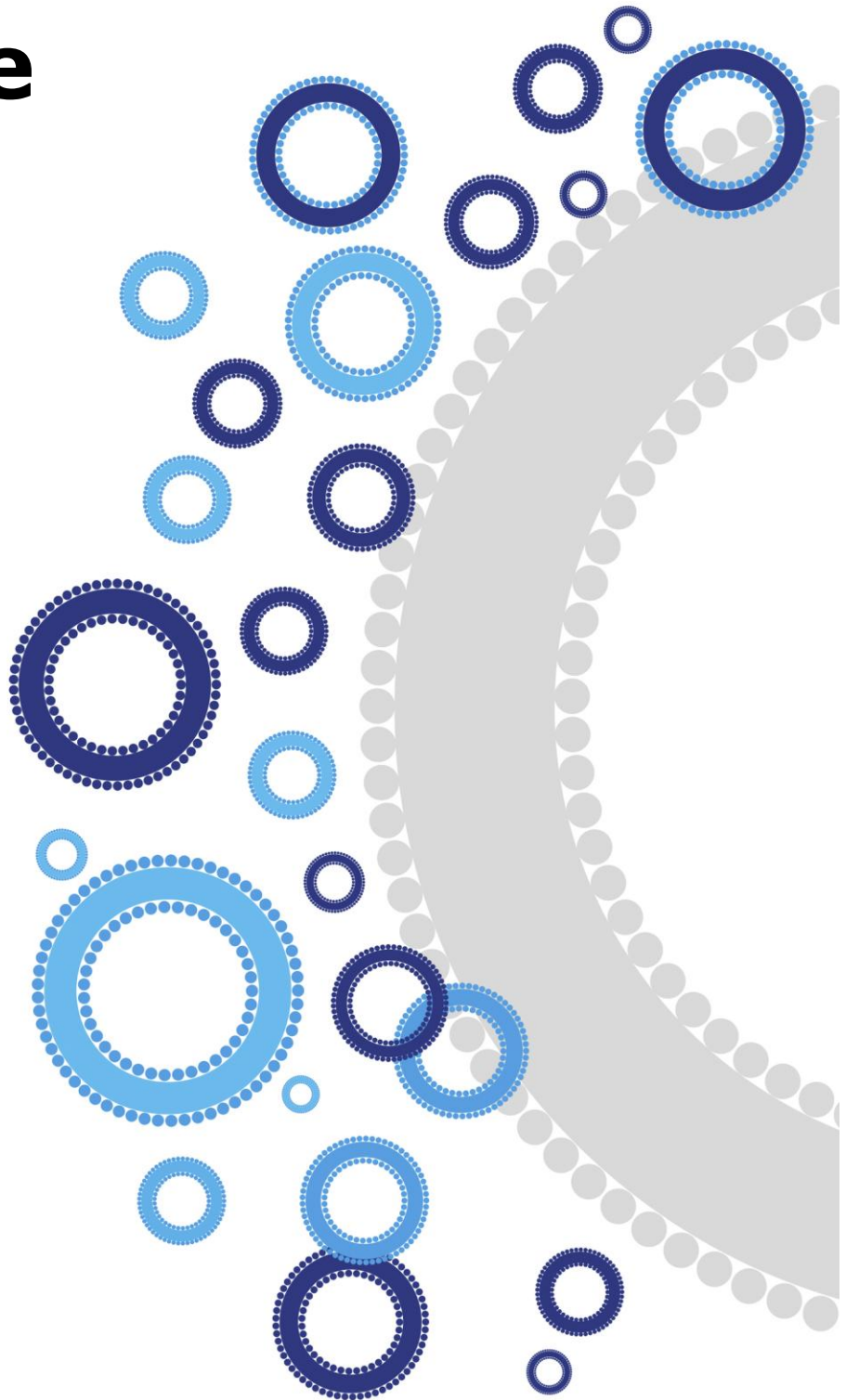


User Guide

EVlution™

**Benchtop Tangential
Flow Filtration**

**Catalogue number
TFF01**



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Introduction

EVlution™ is a compact tangential flow filtration (TFF) system designed for efficient size-dependent concentration and purification of extracellular vesicles (EVs) and other molecules.

TFF provides an effective method of size-based separation of EVs from other biomolecules, whilst minimizing membrane fouling. The characteristics of TFF design makes it an excellent choice for workflows at the lab scale.

The adaptable design of EVlution™ supports a wide range of hollow fiber filter configurations, making it a flexible solution for diverse laboratory-scale applications. With an intuitive interface and streamlined operation, EVlution™ simplifies complex filtration processes while delivering reliable performance.

Applications of EVlution™ include:

- Concentrating and purifying EVs
- Depletion of EVs from FBS
- Concentrating and purifying proteins, peptides, and nucleic acids.
- Recovering proteins, antibodies, and extracellular vesicles (EVs) from cell culture media.

Important Notice

This equipment is intended for professional laboratory research use only. Before installation or operation, ensure the instructions provided in this manual have been reviewed.

1. Install the instrument in a clean, dust-free, non-hazardous, and well-ventilated area (e.g. class 2 biosafety cabinet, room temperature laboratory bench)
2. Never use the instrument with any corrosive or combustible liquids. Avoid using flammable liquids unless when cleaning the system (e.g. 70% ethanol)
3. Overpressure or viscous liquids can cause the tubing to burst / disconnect from the system
4. Before each use, check for kinks or restrictions in the tubing. This may also cause the tubing to burst / disconnect from the system
5. Pump tubing should be replaced intermittently as per the manufacturer's guidelines.
6. Do not move the system while it is operating
7. In case of any issues with instrument, please contact tech@cellgs.com
8. Please discard packing materials in accordance with relevant local regulations.
9. Visit www.cellgs.com to refer to the latest product guide for more information.
10. Operating Conditions:
 - a. Ambient temperature: 15~ 25°C
 - b. Relative humidity: 80% RH Max.
 - c. Power supply: DC24V 1.9A
 - d. Altitude: up to 2000 m
 - e. Indoor use



Do NOT power on the system until all setup steps have been completed and verified



Do NOT operate above the recommended pressure as this can lead to damaged components or cause tube disconnection.



Turn off the system before making any adjustments to the equipment set up

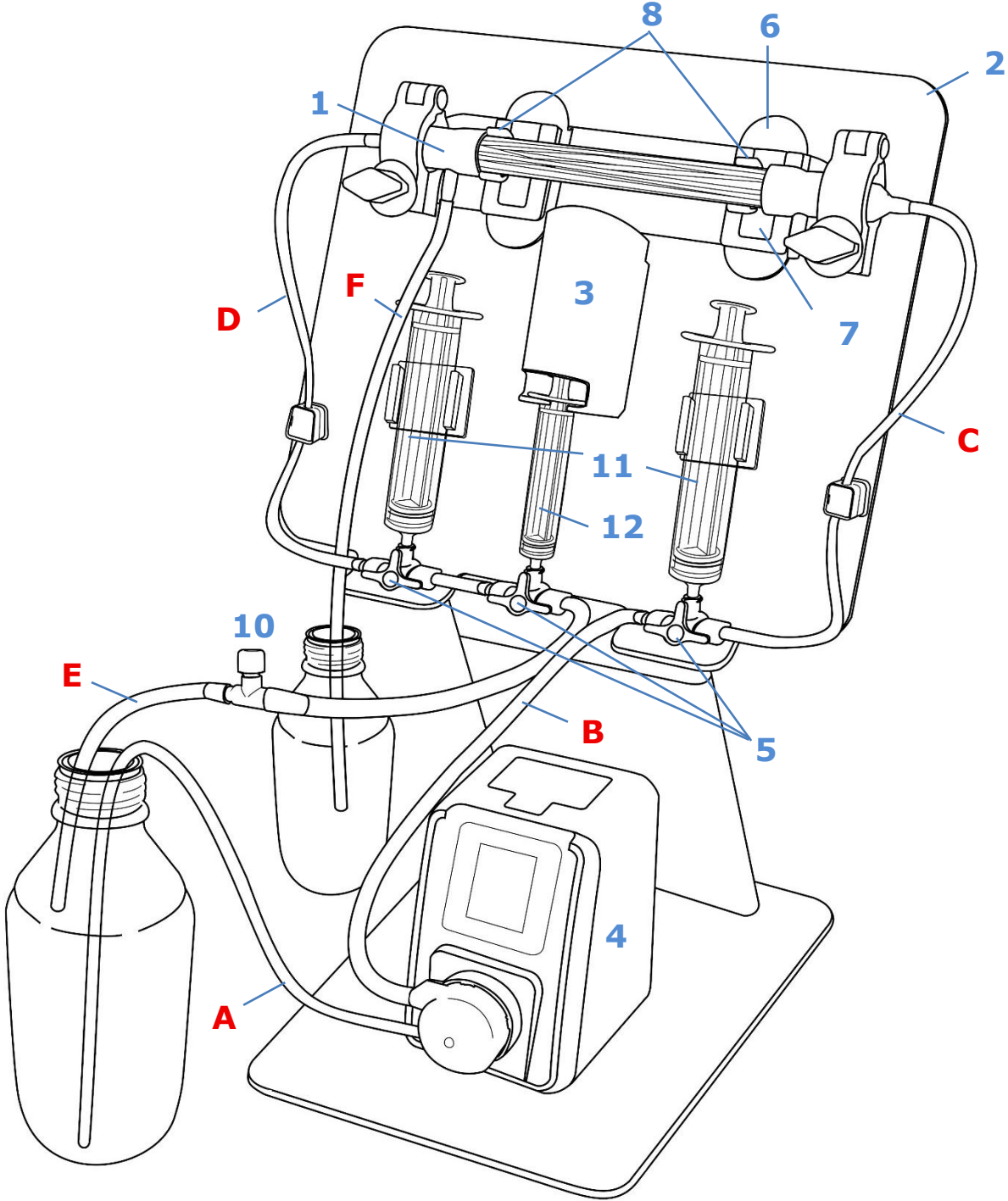


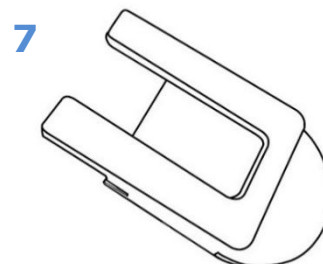
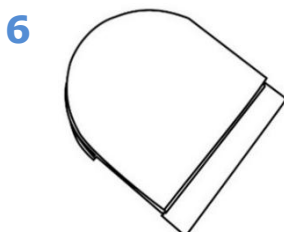
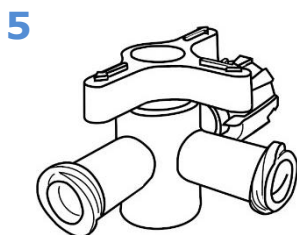
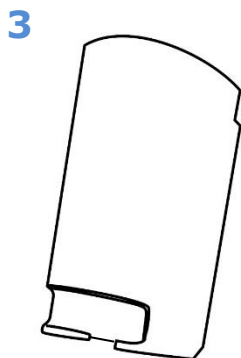
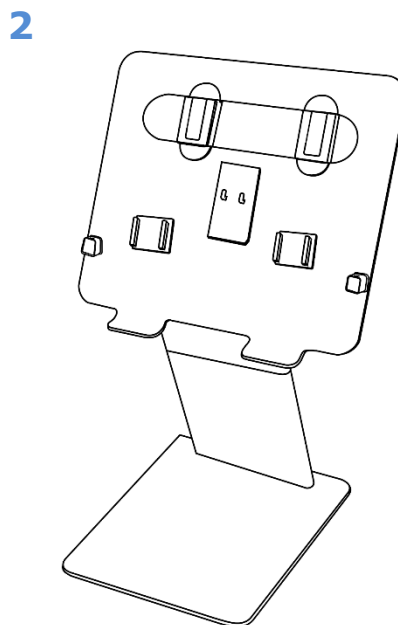
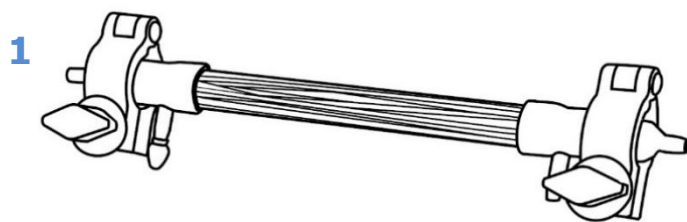
Wear proper PPE (**lab coat, gloves, and safety glasses**) at all times to prevent injury or exposure to hazards.

Storage

All components should be stored at room temperature (15°C - 25°C).

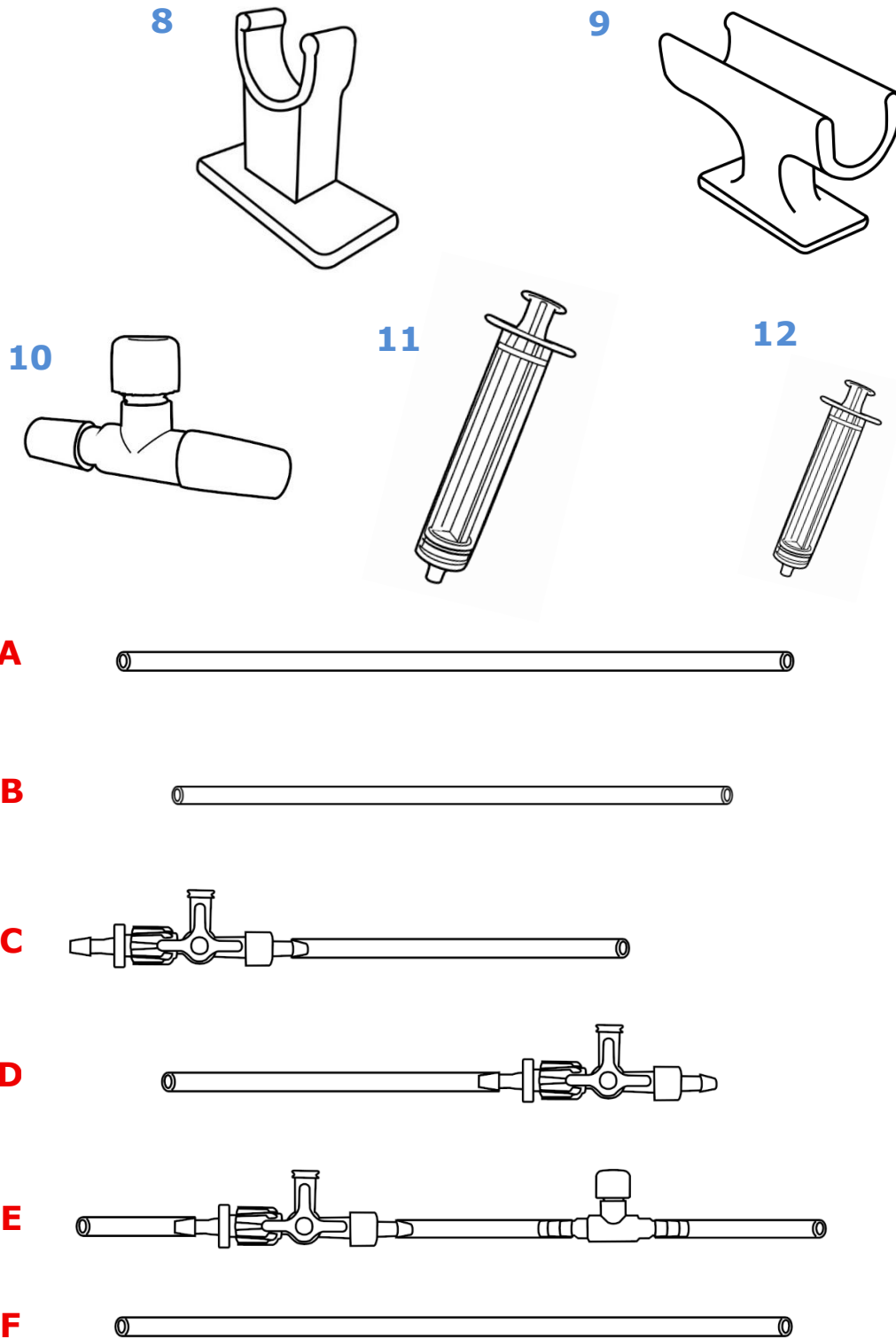
Product Components





Package Includes:

1	EVlution™ hollow fiber filter
2	EVlution™ stand
3	Pressure gauge syringe housing
4	EVlution™ peristaltic pump (pedal and cable included)
5	3-way stopcock (x3) (male and female luer locks attached)
6	Upper cartridge mount (x2) (Attached to the stand)
7	Lower cartridge mount (x2) (Attached to the stand)
8	Horizontal filter clip (x2)
9	Vertical filter clip
10	Flow control valve
11	20 mL disposable syringes (x2)
12	5 mL disposable syringe
A-F	Tubes A-F (supplied assembly)



Note: A-F show the preassembled tubing and valve parts provided, consisting of components from the equipment list. Tubing is depicted for illustrative purposes only; length is not to scale.

Package does NOT include: retentate and permeate containers (e.g. Duran bottles)

Product Information

EVlution™ may be used for concentrating EVs, such as exosomes, from cell culture medium and other biofluids, such as urine. Other bodily fluids, such as cerebrospinal fluid and breast milk have not been tested at the time of writing. The protocols outlined in this booklet can be adapted for these and other sample types, such as proteins. The recommended range of starting material is 100 mL to 1 L.

Concentrated and purified EVs generated with EVlution may be further purified, using our widely-cited Exo-spin™ size-exclusion chromatography columns and characterized using our range of EV assay kits and reagents.

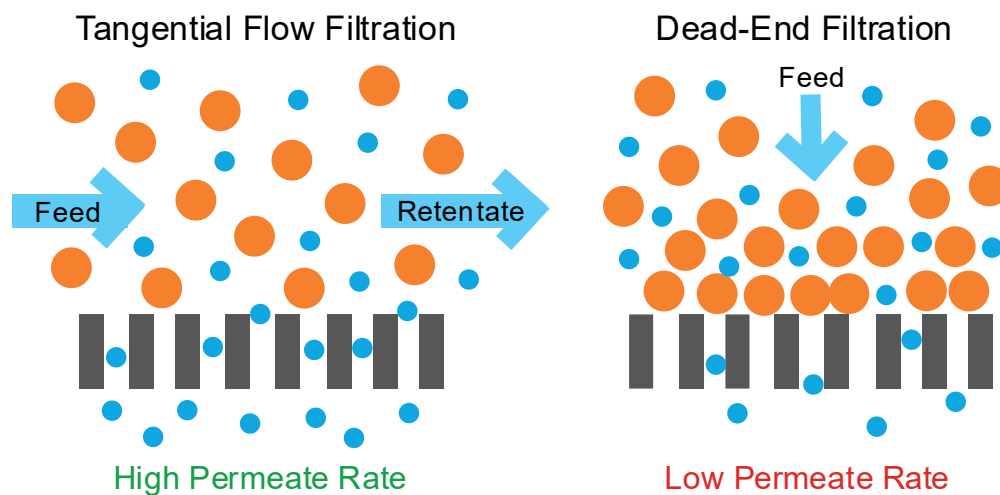
General Information

How EVlution™ works

EVlution™ uses Tangential Flow Filtration (TFF) to separate and concentrate samples. In TFF, the liquid flows tangentially across the membrane surface, which helps prevent clogging. As the sample moves along the membrane it is divided into:

- **Permeate** – the portion of the fluid that passes through the membrane, containing smaller molecules – is removed.
- **Retentate** – the portion of the fluid that is retained by the membrane, containing larger molecules – becomes increasingly purified and concentrated.

By maintaining continuous tangential flow, EVlution™ enables efficient concentration, while preserving membrane performance.



In dead-end filtration, the fluid is pushed directly into the membrane surface, causing particles to accumulate quickly. This leads to rapid filter clogging, lower throughput, and more frequent interruptions.

In contrast, TFF keeps the fluid moving across the membrane rather than straight into it. This tangential flow continually clears the membrane surface, resulting in:

- less fouling
- more consistent filtration performance
- longer membrane life

EVlution™ uses this tangential flow approach to deliver reliable, repeatable concentration and separation from non-EV sample components with minimal user intervention.

Sterile vs non-sterile conditions

The EVlution™ system can be used in both sterile and non-sterile conditions. For non-sterile conditions, set up the equipment on a suitable worktop as per the instructions in the *Assembly* section.

For sterile conditions, set up the EVlution™ in a class 2 biosafety cabinet. Spray or perfuse each component with 70% ethanol. As part of the cleaning protocol (see *Operation Protocol* – section C *System Cleaning and flushing*) the whole system is also flushed through with 70% ethanol to aid in sterilization.

All the components of the EVlution™ system that come into contact with the sample, including the syringe-based pressure gauge, are disposable.

Peristaltic Pump



1	Color LCD display
2	Pump head
3	Pump control buttons
4	Speed control knob
5	Foot switch interface
6	DC24V power output
7	DC24V power input

Button functions

Buttons	Designation	Description
Menu	Menu button	Enter the menu, or return from a sub menu
-	Up button / decrease the value	Decrease the rpm, or scroll up in a menu screen
Set	Set button	Enter the setting menu, or return to the previous menu
DIR	Pump head direction	Switch the pump running direction
+	Down button/ increase the value	Increase the rpm, or scroll down in a menu screen
Max	Maximum speed button	Run the pump at full speed
▶/ 	Start/ stop button	Start or stop the operation



Display



Position	Description
1	Indicates the running direction of the pump
2	Indicates whether the operation is running
3	The pump is on continuous mode
4	The time the current operation has taken
5	The volume that has passed through the pump in the current operation*
6	Run the pump at full speed

***Note:** The volume shown on the display reflects how much liquid has passed through the pump, not the total amount of sample that has been processed or filtered. Because the system recirculates, the same fluid may pass through the pump multiple times, so the displayed volume will be higher than the actual sample volume.

Assembly

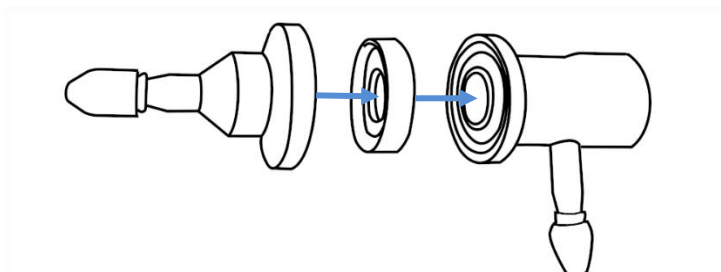
For more guidance, please see our video tutorial [here](#), or scan the QR code. The video can also be found on our website at www.cellgs.com and <https://www.youtube.com/@CellGS>.



EVlution™ cartridge assembly

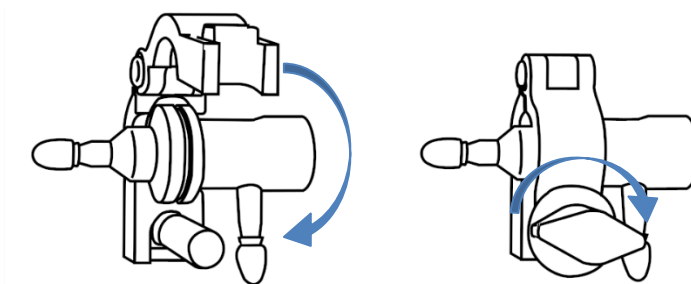
Install the O-Ring and Tube Adapter

1. Slide the **rubber O-ring** onto the end of the hollow fibre cartridge.
2. Ensure the O-ring is seated evenly and is not twisted.
3. Position the **tube adapter** over the O-ring.
4. Press the tube adapter firmly into place until it is fully seated.



Position the Cartridge in the Clamp

5. Place the cartridge end (with the adapter installed) into the **clamp**.
6. Fold the clamp down until it locks securely.
7. Twist the **screw cap** clockwise onto the threaded part of the clamp.
8. Continue tightening until the cap is firm and secure (hand-tight).

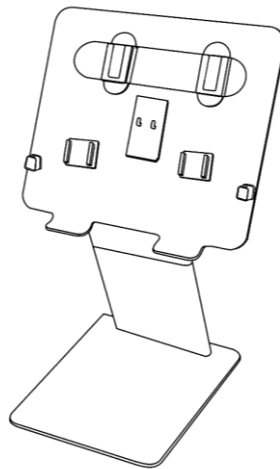


9. Repeat on the other side. Ensure that the screwcaps are facing the same side.

EVlution™ system assembly

Note: If required, EVlution™ can be set up within a biosafety cabinet. Each component can be sprayed with 70% ethanol, prior to assembly.

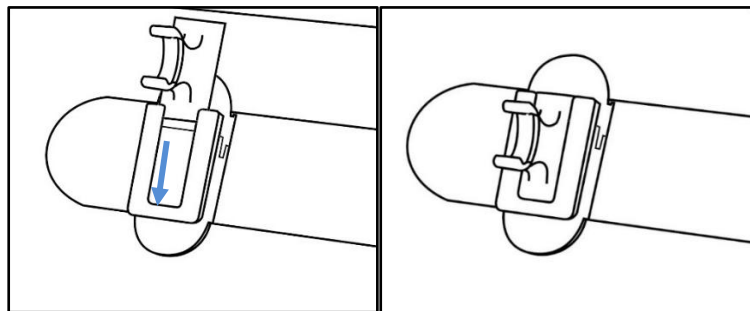
1. Carefully remove all the outer packaging for all of the components.
2. Set up the EVlution™ stand so that it is upright and extended from the base stand. This may require a small amount of force when first assembled.



3. Slide the filter clips into the brackets. For the horizontal orientation follow section A. For the vertical orientation follow section B.

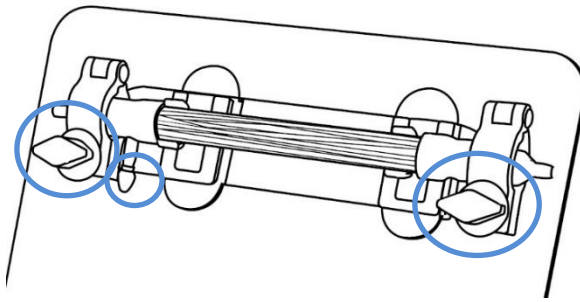
A. Horizontal cartridge orientation

1. Slide the horizontal filter clips (x2) into the bracket mounts on the front side of the stand. Ensure each clip is fully seated and secure.



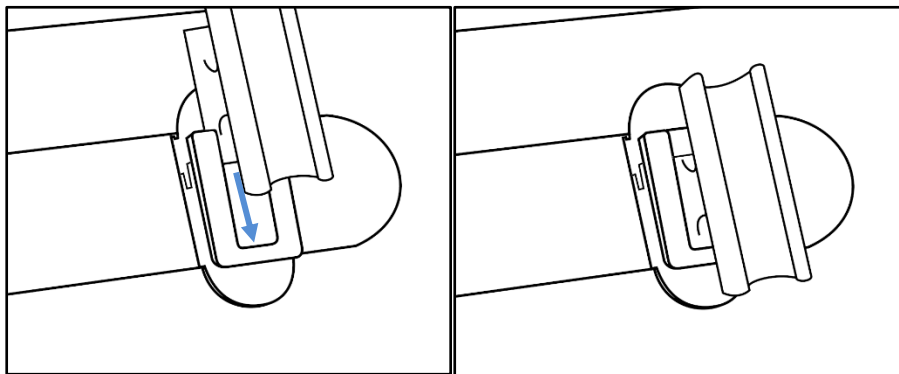
2. Attach the cartridge to the filter clip. Ensure that the screw caps on the filter cartridges are facing outward. Remove the red rubber stoppers from the sides of the filter, and from the left permeate outlet. Leave the stopper on the right permeate outlet

Note: The cartridge may have condensation inside – this is normal



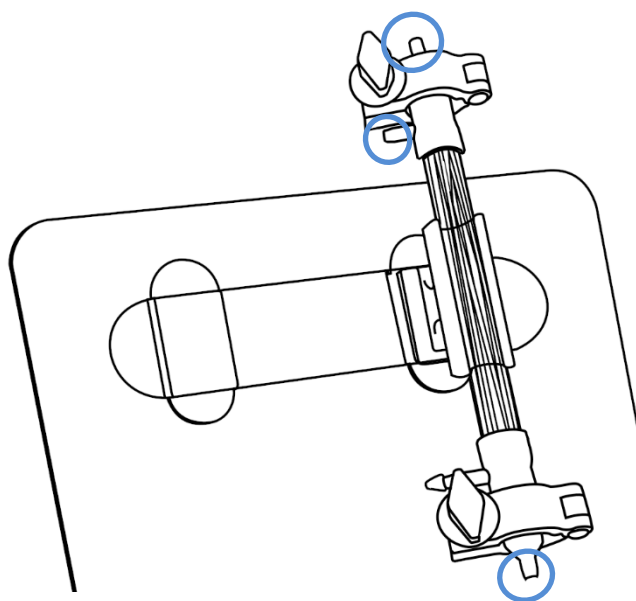
B. Vertical cartridge orientation

1. Slide the vertical clip into the double-sided bracket mount on the rear side of the stand.



2. Attach the cartridge to the filter clip. Remove the red rubber stoppers from the sides of the filter, and from the top permeate outlet. Leave the stopper on the bottom permeate outlet

Note: The cartridge may have condensation inside – this is normal



4. Connect the **pedal cable** to the peristaltic pump.



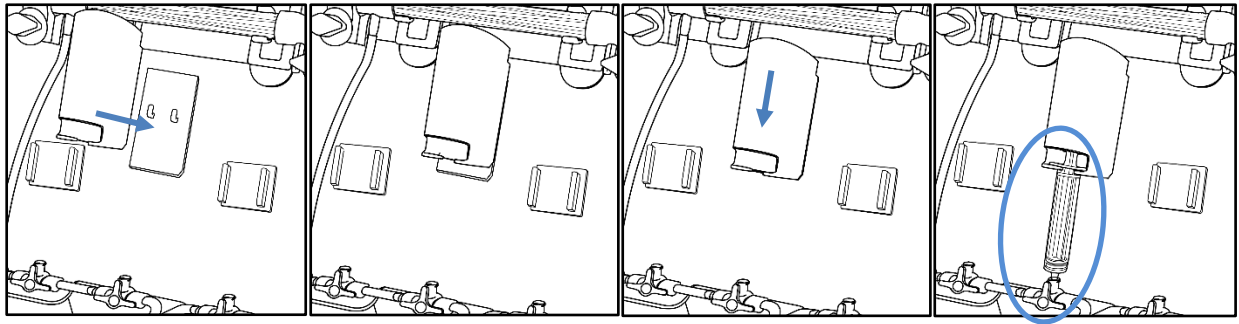
5. Connect the **power cable** to the peristaltic pump. Plug in the power cable.



6. Place the pump onto the base of the stand, directly below the filter membrane.
7. Attach the pressure gauge of choice to the stand. For the syringe pressure gauge follow section A. For the digital pressure gauge follow section B.

A. Syringe pressure gauge

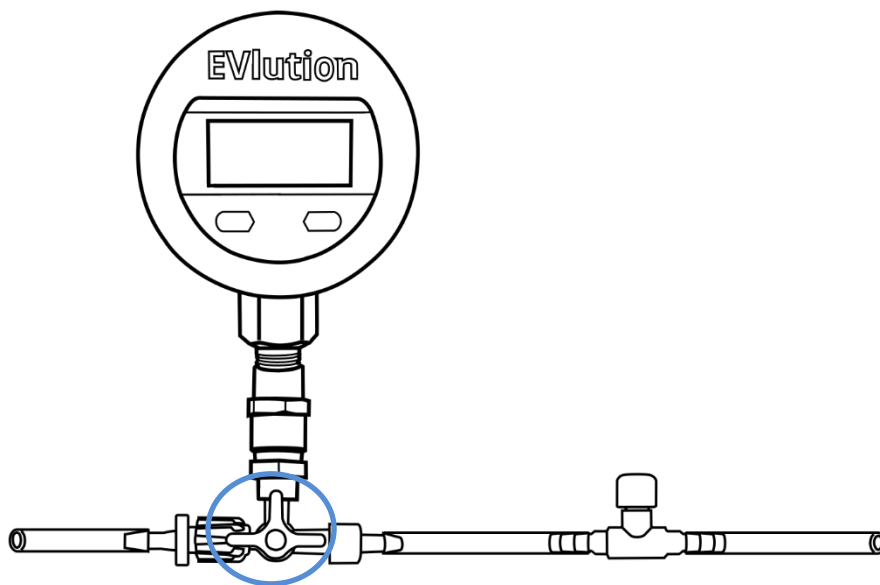
1. Slide the pressure gauge syringe housing onto the mounting plate. The holes on the back of the syringe housing line up with the hooks on the mounting plate. Slot the hooks into the holes, then gently push the housing down.



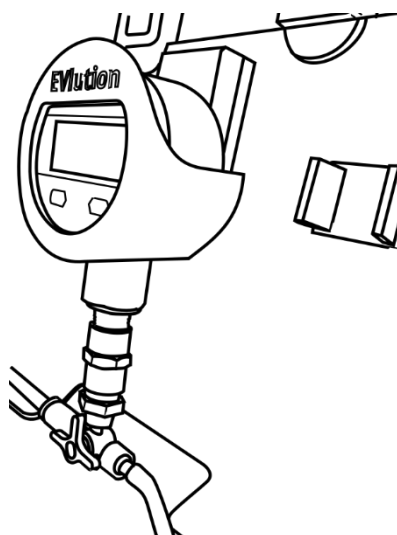
2. Fit the 5 mL syringe into the housing and connect the end of the syringe into the central 3-way stopcock.
3. Attach tube A to the inlet of the pump. The other end of tube A should be placed into the retentate container.
4. Continue to step 9 of the *EVlution™ system assembly*

B. Digital pressure gauge

1. Slide the digital pressure gauge into the housing.
2. The luer lock connector is twisted into position by rotating clockwise to attach the digital pressure gauge.
3. Take tube E and connect the white adaptor to the stopcock. Twist until secured. Ensure the stopcock lever is facing forward.



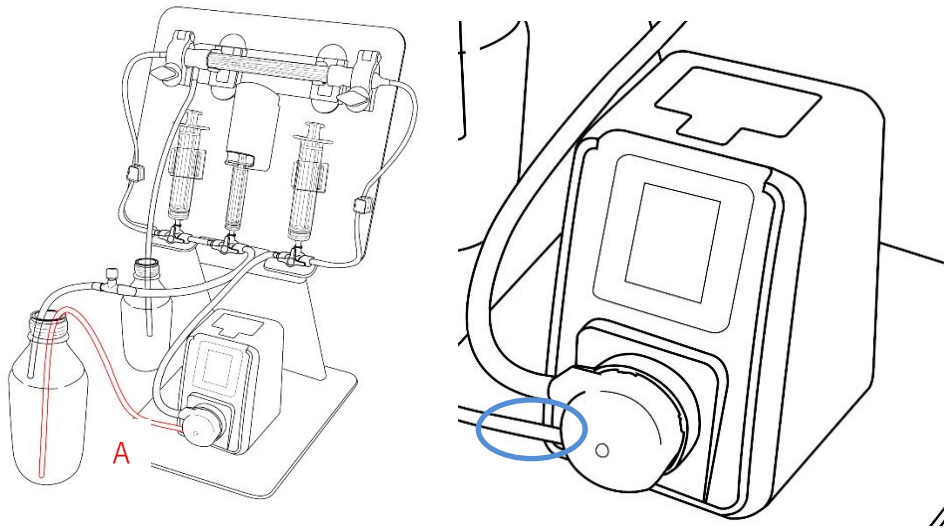
4. Align the holes in the back of the gauge holder with the hooks on the mounting plate. Slot the hooks into the holes, then gently push the housing down



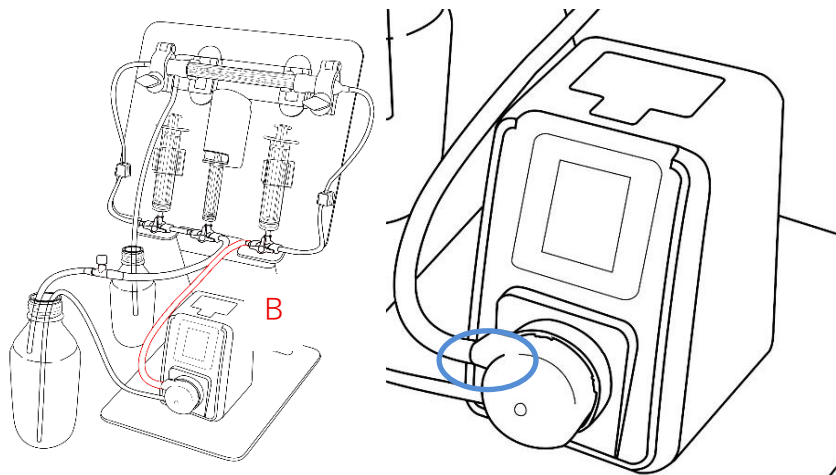
5. Long-press the UNIT button to power the gauge on.
6. Select the required pressure unit by short-pressing UNIT button. BAR is recommended for EVlution™ use.
7. With the system at atmospheric pressure (pump off, pressure control valve open), long-press ZERO for 5 seconds to zero the gauge.
8. The gauge is now ready for use. Continue to step 9 of the EVlution™ system assembly

For more information on the digital pressure gauge, please see the *Digital Pressure Gauge* user guide at www.cellgs.com.

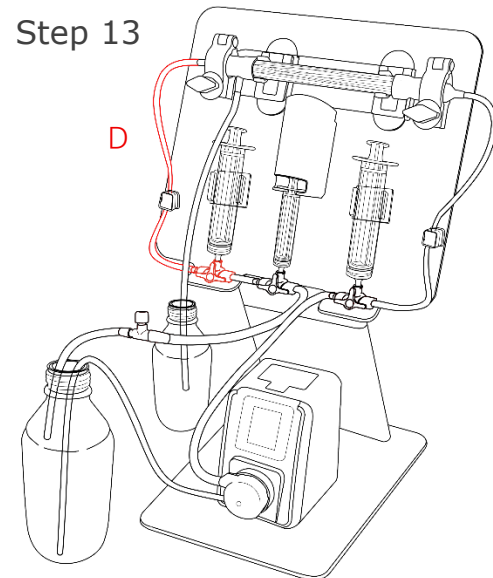
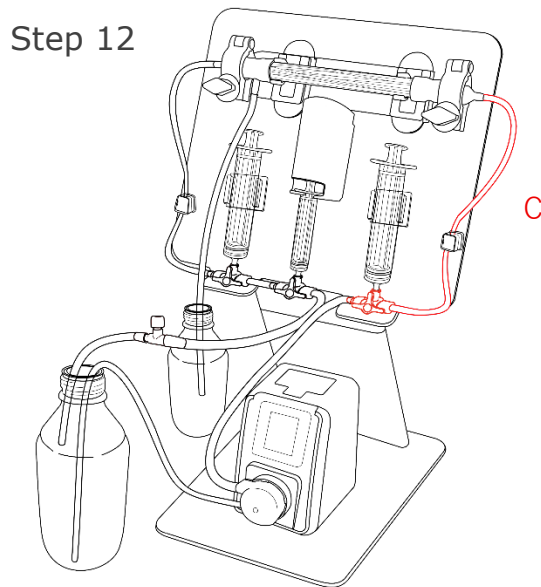
9. Attach tube A to the inlet of the pump. The other end of tube A should be placed into the retentate container.



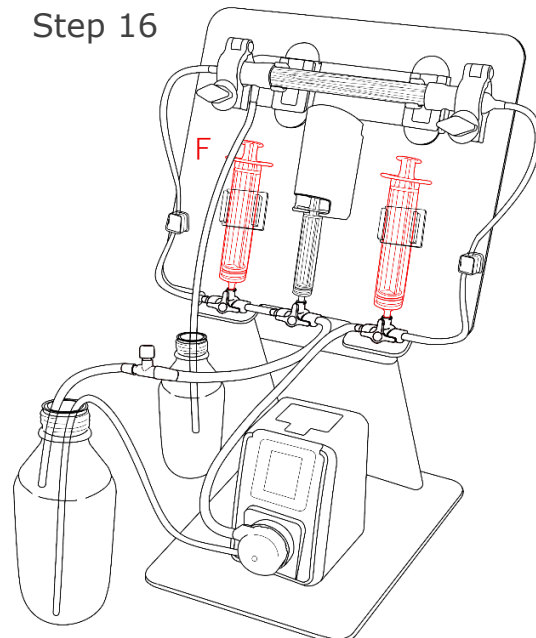
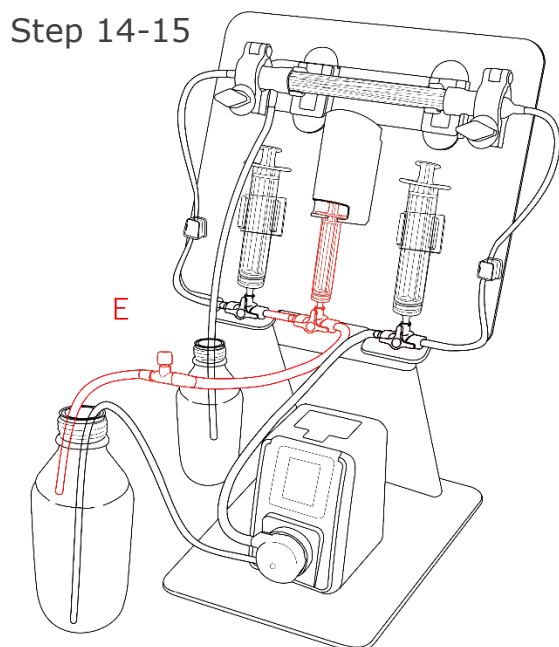
10. Attach tube B to the outlet of the pump.



11. Attach tube C's three-way stopcock to the end of tube B, then attach the other end to the cartridge. **Vertical orientation:** attach the tube end to the bottom end of the cartridge
12. Attach the tube part of tube D to the other end of the filter membrane. **Vertical orientation:** Attach the tube to the top end of the cartridge.



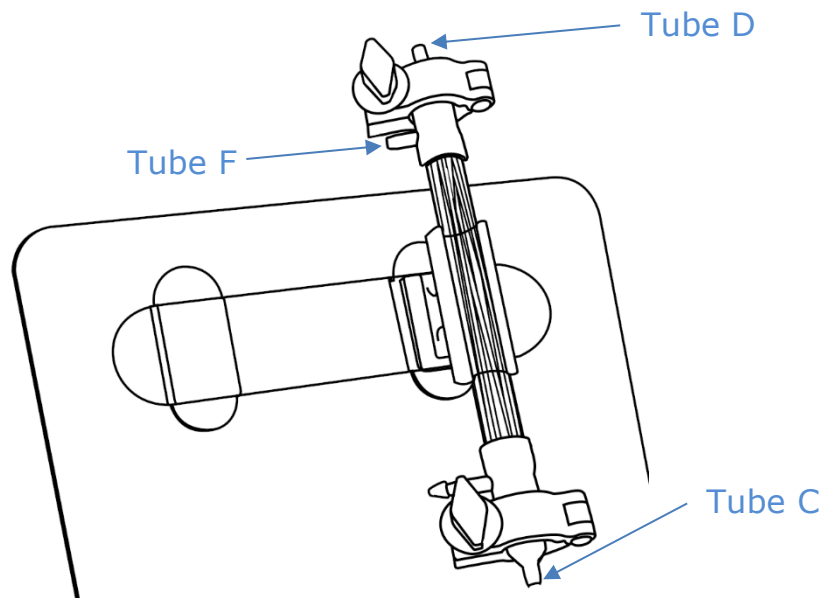
13. Attach the short tube end of tube E to the three-way stopcock of tube D. Attach a clean, 5 mL syringe to the top tube E's three-way stopcock.



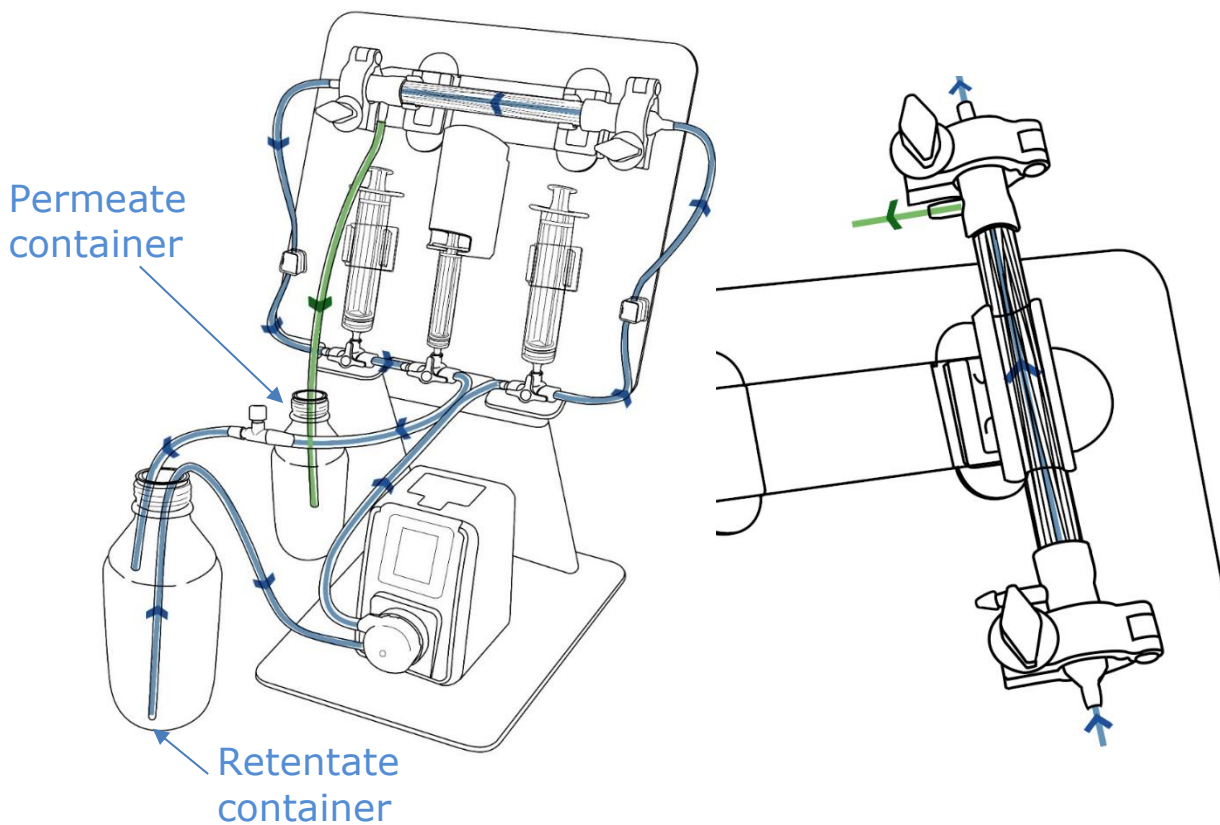
14. Place the other end of tube E (retentate tube) into the retentate container. **Vertical orientation:** Attach the tube to the top permeate outlet (the bottom

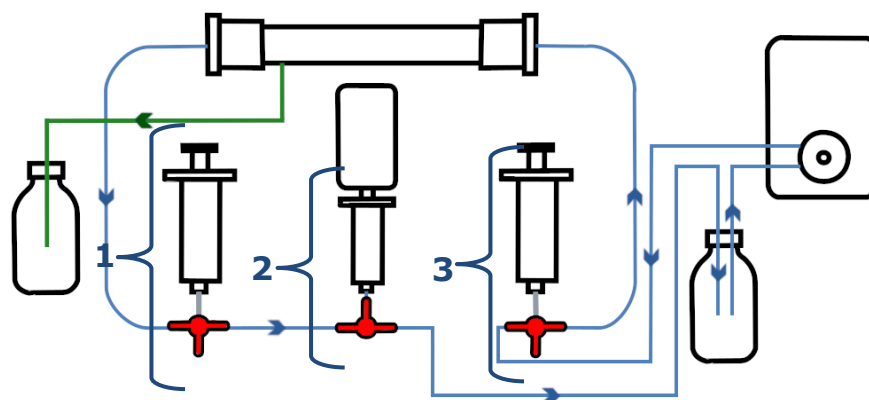
- outlet should still be plugged with a red rubber stopper)
15. The 20 mL syringes may be installed at this stage. Alternatively, the syringes may be added later, as described in Operation protocol - section C.
 16. Attach tube F to the left permeate outlet.

Vertical cartridge orientation tube attachment diagram



The completed set up and flow pattern of the EVlution™ system





- Blue path: the flow of the sample entering through the inlet, across the TFF cartridge and looping back as retentate
- Green path: A portion of the sample passes through the membrane and exits separately as permeate.
- The syringes and three-way stopcocks will be referred to as syringe 1, stopcock 1, etc.

Peristaltic Pump Calibration

1. Go to **Menu > Calibrate**.



2. Select **Empty** to fill the system with water.
3. Press **Set** to start filling, wait until full, then press **Set** again to stop. The screen will switch to the calibration interface.
4. Place the output tube in an empty measuring cylinder.



5. Use **+** and **-** buttons to set the calibration time.
6. Press **Set** to start calibration. The pump will run for the set time and stop automatically.
7. Measure the water collected. Enter this volume using **+** and **-**, then press **Set** to confirm.

The pump is now calibrated.

Pedals

The default setting of the foot pedal is to start/stop the pump. This can be changed via the system menu.



1. **Pedal**: Title of the screen, when the selection highlight is on this option, press the **Set** button to return to the previous menu
2. **Trigger**: Use the foot switch to control the pump: press once to start, press again to stop
3. **High Level**: This changes the switch control to stop the pump when the switch is pressed and starts the pump when the switch is released.
4. **Low Level**: This changes the switch control to start the pump when the switch is pressed and stop the pump when the switch is released.

Operation Protocol

A. Important notice before starting

There are a variety of procedures that need to be followed during different phases of EVlution operation. Please follow the instructions for system set-up.

- Confirm all fittings and tubing are securely connected before pumping the liquid
- Do not adjust tube connections whilst the system is in operation. If adjustments are needed, stop the pump first and flush through with air before attempting to disconnect tubing. To reconnect any tubes, ensure that the pump has stopped first.

The general workflow is outlined below:



Note: It is recommended to calibrate the pump before first use. Afterwards, the pump should be calibrated before using a new cartridge.

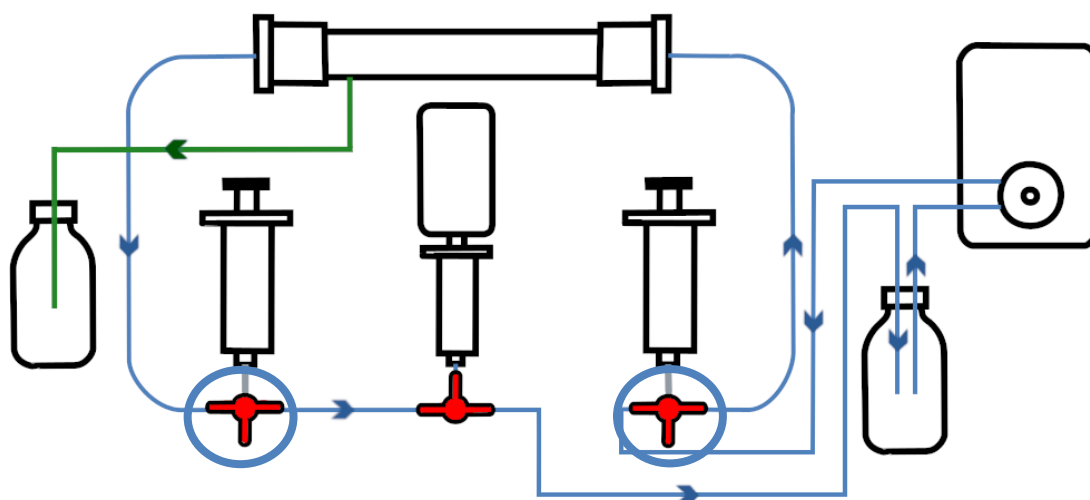
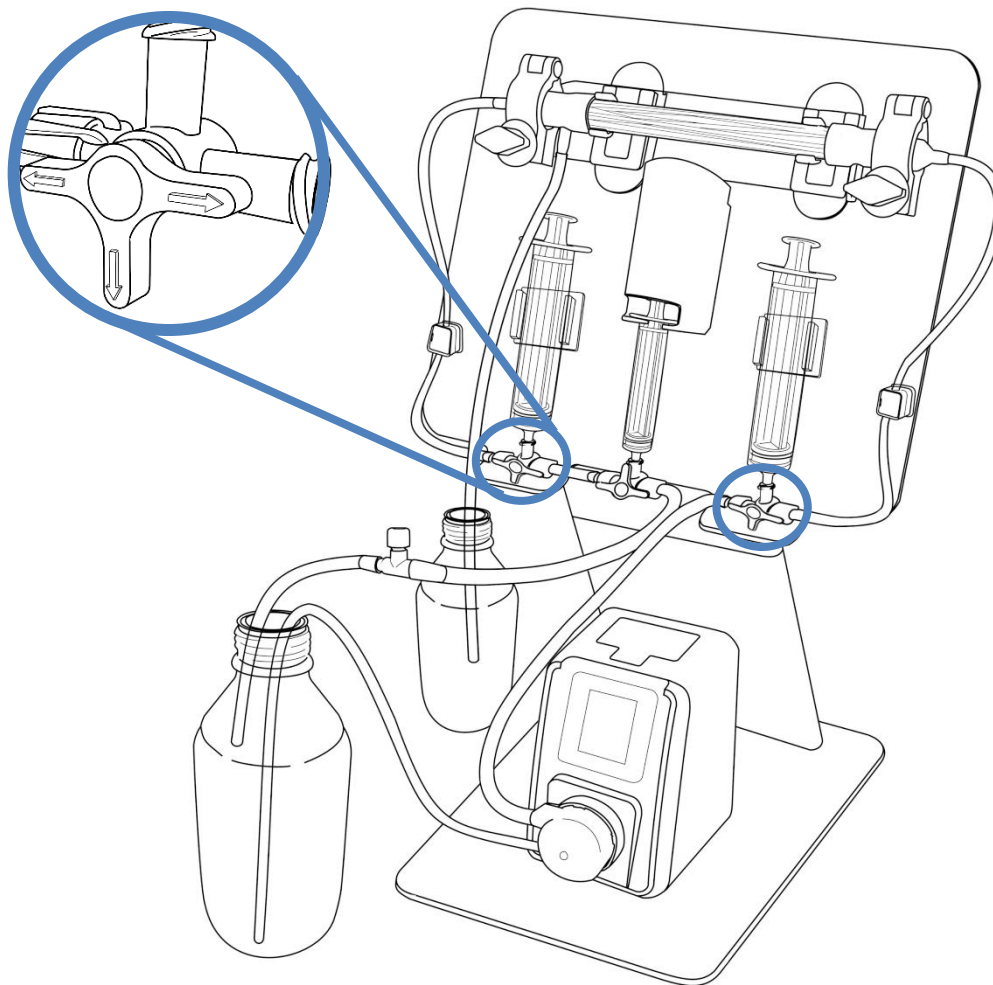
B. Configuring the system: Closed loop and hands-on modes

There are two operation modes, and the transition from one to the other is performed via a process referred to as Switch Flow™:

1. Closed loop mode (automated, circulating) is used to reduce large volumes. In the closed loop phase, liquid flows in a continuous cycle through the TFF. This mode is used for bulk reduction of sample volume down to 20 mL.
2. Hands-on mode (manual, back and forth). The hands-on mode is for concentrating samples further (e.g. from 20 mL to 10 mL).

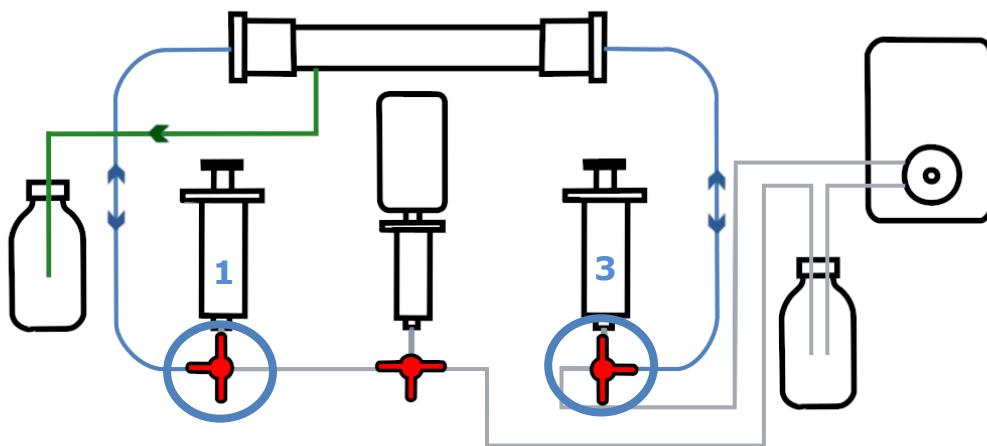
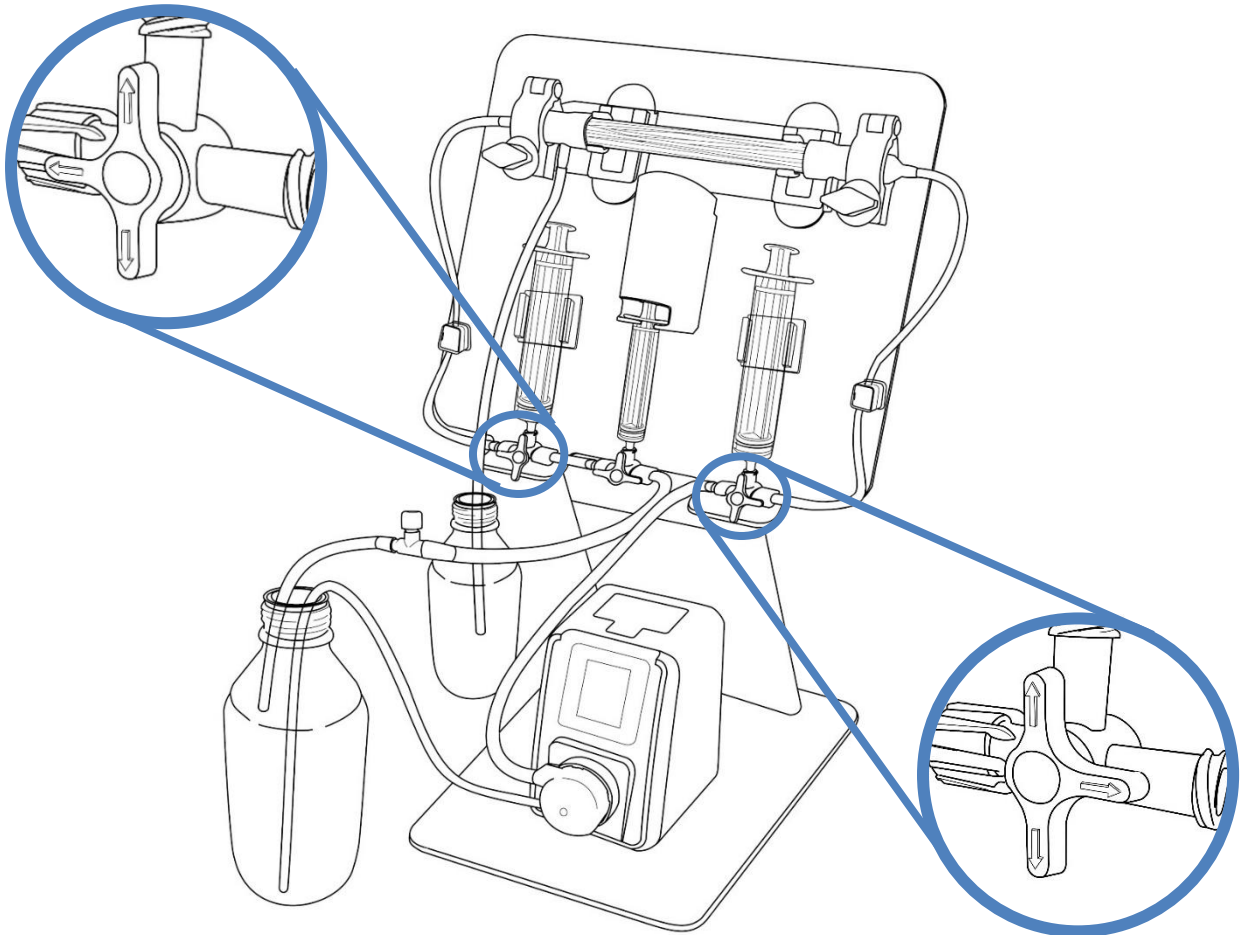
Closed loop mode

The system has three three-way stopcocks. The two outer stopcocks (stopcocks 1 and 3) should be turned so that they are pointing downward. This will ensure that liquid can pass through, but not up into the syringes. The central stopcock (stopcock 2) should point upwards such that the pressure syringe is connected to allow pressure to be recorded.



Hands-on mode

The two external stopcocks (stopcocks 1 and 3) should be turned so that they are pointing away from each other. This will ensure that liquid can only pass through the filter and the syringes on either side.



C. Sample Concentration Part 1 (e.g. 1 L to ~10 mL)



For more guidance, please see our video tutorial [here](#), or scan the QR code. The video can also be found on our website at www.cellqs.com.

This section describes how to concentrate an input volume of up to 1 litre down to 20 mL using loop mode and then to 10mL using the hands-on (back and forth) mode.

Note: An initial working volume between 100 mL and 1 L is recommended. While larger volumes of up to 2L can be processed, using volumes below 50mL is not recommended.

Preparation of the sample

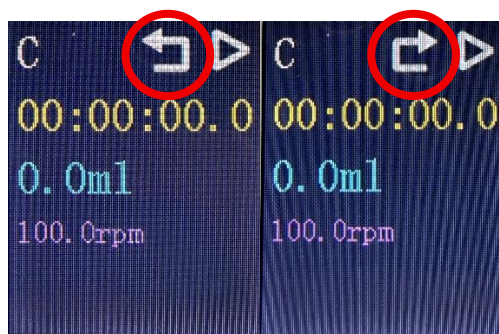
Biological fluids contain cells and large cellular debris which may be co-purified with the EVs in the retentate. These should be removed before the sample is subjected to TFF. To remove cell debris, centrifuge the sample at $500 \times g$ for 5 minutes and collect the supernatant. This will be the starting material.

Vertical or Horizontal Cartridge mode?

We have obtained good results when using the cartridge in a horizontal position. This is most convenient for the Switch Flow™ mode (see below) to reduce the final retentate volume. However, if you find that pressure is drifting upwards during use, this may indicate that bubbles are accumulating on the inside of the hollow fibres reducing the effective surface area. Positioning the cartridge vertically allows these bubbles rise and escape. The cartridge may then be moved to the horizontal position during Switch Flow™ mode.

Circulating the sample

1. Confirm that the EVlution™ system is set up as described in the *Assembly* section.
2. Fill the retentate container with the sample liquid.
3. Ensure the system is in the closed-loop mode.
4. Check that the flow control valve is open. Twist the knob **anti-clockwise** by two turns to be sure.
5. Set the pump to 250 rpm.
6. Ensure the pump flow is set to **anti-clockwise**. This is indicated by an arrow icon on the pump screen. To change the direction, press the "DIR" button and this should change.



7. Press down the pedal or the ►/ || button to start the pump.
8. Allow the liquid to circulate through the system.

Initiating permeate flow

9. Once the liquid begins exiting the retentate tube, pressure can be applied to begin filtration. This is done by controlling the pressure with the control valve.
10. Turn the control valve clockwise **VERY** slowly, to begin increasing pressure. Eventually, the syringe pressure gauge will begin to move upwards. It is recommended to use a pressure of 1 bar, indicated by 1 mL.

Note: If the syringe exceeds 1.5 bar (1.5 mL on the syringe), the tubing may disconnect: decrease the pressure immediately by opening the flow control valve. Release the pressure by twisting the knob of the control valve **anti-clockwise**. Alternatively, stop the operation by pressing the pedal switch or the ►/ || button. Once the pressure syringe is back to the default position (zero mL), the operation can be restarted.

11. Once the pressure has been set, the permeate should begin to flow from the filter and exit through the permeate tube. When this has been confirmed visibly, the EVlution™ system can be left to run independently.

Note: The time required for filtration depends on the cartridge fitted, the sample type, sample volume, fluid pressure and flow rate. Inhouse test show that it typically takes 90 minutes to concentrate a starting volume of 1 litre of 1% FBS down to ~20 mL.

12. When the volume remaining in the retentate container reaches <10 mL, and before bubbles start to be introduced into the circulating liquid, stop the pump.

Note: The residual within the system will be ~20 mL, giving a final volume of <30 mL

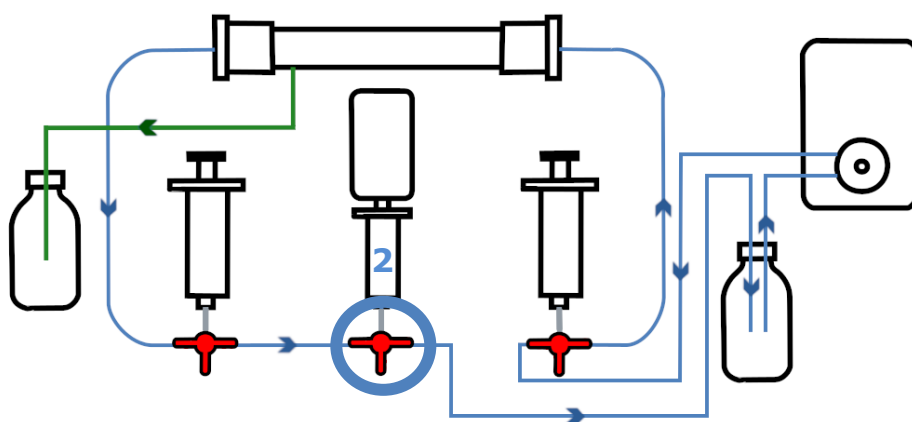
Finalising or continuing concentration

If the sample requires further concentrating to reduce volume, proceed with the steps below for manual final processing. If the volume is already small enough, continue to Operation protocol - section D. *Sample transfer, Without Switch Flow™*.

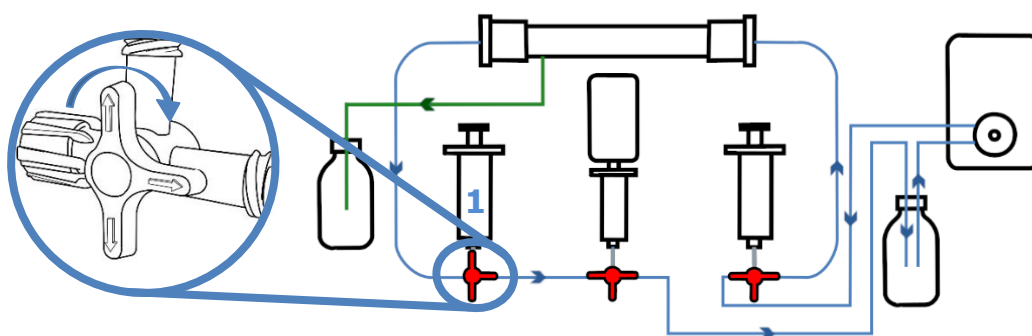
Further concentration via the hands-on Switch Flow™ mode.

This section describes how to concentrate an input volume of ~20 mL down to <10 mL using the syringes to manually pump the solution across the TFF cartridge. The final sample is collected in one of the 20 ml syringes.

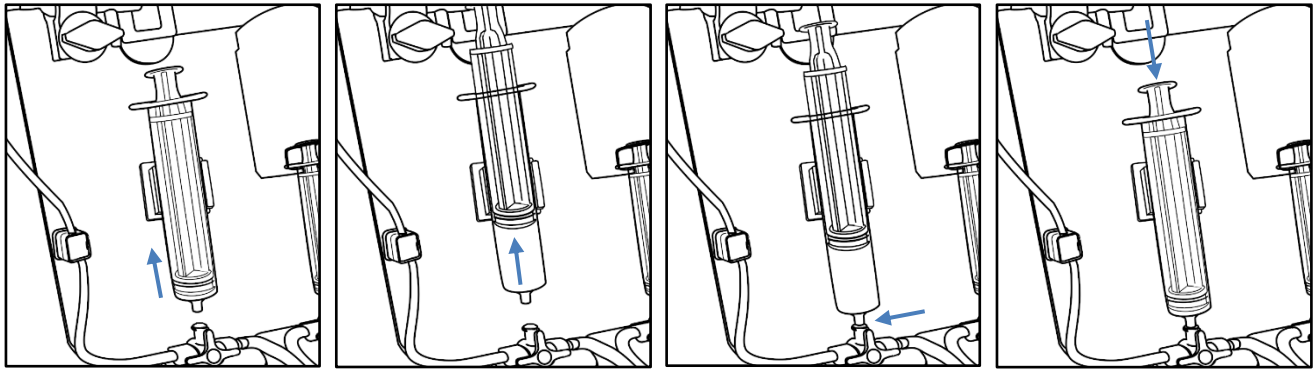
13. Turn stopcock 2 attached to the syringe pressure gauge so that it faces downward, and the liquid is able to bypass the syringe itself.



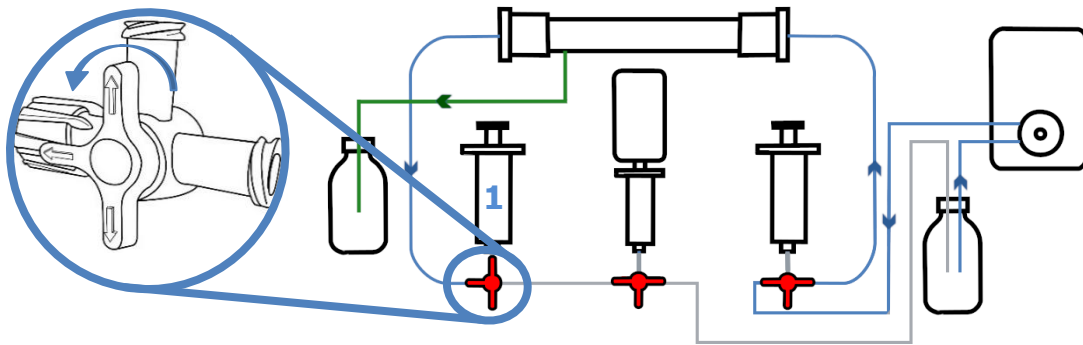
14. Turn stopcock 1 so that it faces right as shown in the image below:



15. Remove syringe 1, take up about 20 mL of air, then connect the syringe to stopcock 1. Push down the plunger to push out the remaining liquid in the tube between the syringe and the retentate reservoir bottle. Take off the syringe and repeat as necessary until all liquid has been pushed out.



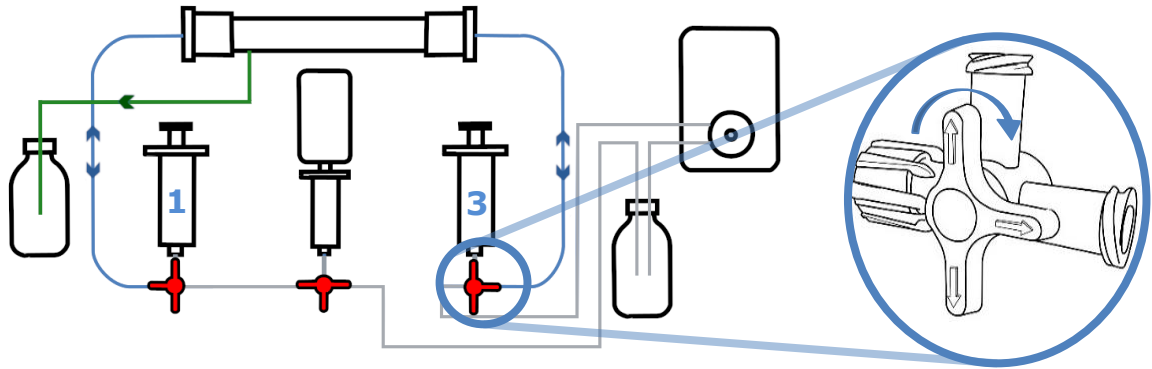
16. Once all the liquid has been ejected, take the syringe out, push the plunger of the syringe all the way down, then reattach it to the three-way stopcock.
17. Turn the handle of the three-way stopcock to face the left, as shown in the image below:



18. It is important for the next step that the total volume of fluid in the retentate bottle is >15 ml. Reduce the speed of the pump to 50 rpm and then allow the pump to run until all the liquid from the container has been taken up. Allow the pump to continue to run to introduce air into the tubing between the retentate and the stopcock 3.

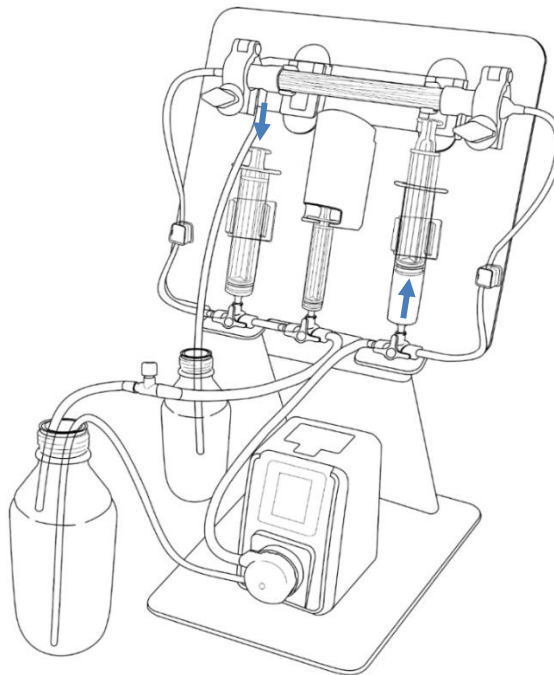
NOTE: When pumping starts, the volume of liquid in the tubing (0.7 mL per 10 cm length) between stopcock 3 plus the liquid in the retentate bottle will displace liquid into syringe 1. Stop the pump before the plunger in Syringe 1 is ejected completely.

19. Once the air reaches stopcock 3, immediately stop the pump, then turn the handle of stopcock 3 to face the right to put the system into hands-on mode.



20. Check that the syringes 1 and 3 are secured to the three-way stopcocks, then alternate pressing down the plunger on each of the 20 mL syringes, in a “see-saw” motion. Liquid should pass through the system, back and forth, with permeate flowing.

Note: Do not lean onto the stand when using hands-on mode.



21. To check the volume, press one plunger all the way down and measure the volume in the other syringe. Once this volume reaches ~5 mL, the total volume remaining in the system will be about 10 mL.
22. Move on to *Section D. Sample transfer*, **With** Switch Flow™

D. Sample transfer

Without Switch Flow™:

Release the pressure by twisting the control valve knob so that it is fully open.

1. Place the retentate tube into the final container of choice.
2. Start the pump and allow all remaining liquid to be transferred through the system into the new container. Once the liquid has been depleted, keep the pump running briefly to push air through the system and ensure complete recovery of the residual volume.

To purify the sample further, it is recommended to use Exo-spin™ SEC columns, also available from Cell Guidance Systems.

3. Proceed to Operation protocol - section E *System cleaning and flushing*

With Switch Flow™:

1. Push the syringe 3 plunger all the way down to begin collecting the liquid in the syringe 1. Turn the stopcock 3 valve to the left then take out syringe 3, draw in air and reattach the plunger to the stopcock 3. Press the plunger down to flush all the remaining liquid into syringe 1. Repeat (if necessary) until all liquid is emptied into syringe 1.
2. Remove syringe 1 containing the sample and transfer the sample into a suitable container (e.g. a 50 ml tube). The sample is now ready for downstream applications or further purification.

To purify the sample further, it is recommended to use Exo-spin™ SEC columns, also available from Cell Guidance Systems.

3. Clean the system as outlined in Operation protocol - section E.

E. System cleaning and flushing

Cleaning the system is recommended after every use. At the start of cleaning, ensure that the input tube remains fully submerged when the pump is on to avoid bubbles, which may affect the effectiveness of the cleaning stage.

Should the input tube rise above the liquid at any time, repeat the current step.

1. Ensure that the EVlution™ is set up as outlined in the *Assembly* section.
2. Set the system to closed-loop mode following the instructions in Operation protocol - section B.
3. Prepare 2 containers each with a minimum volume of 150 mL:
 - distilled water
 - 70% EtOH
 - And one container of 350 mL, 0.5M NaOH

4. Perform a water flush: start by placing the retentate and input tubes into the distilled water container, and the permeate tube into a waste container.
5. **Ensure the flow control valve is fully open before starting the pump at 250 rpm.** The water should circulate in a continuous loop
6. Allow the system to circulate for at least 5 minutes. This marks the end of the water flush step.
7. Stop the pump. Move the retentate and input tubes into the 0.5M NaOH container. Start the pump again and let the NaOH flow in a continuous loop. Then adjust the pressure to 1 bar. Allow this to run for 30 minutes. The following steps should be performed at 1 bar unless specified otherwise.
8. During this time, replace the water in the water container with fresh distilled water. Once the NaOH wash has completed, stop the pump and place the retentate and input tubes into the water container. Perform a water flush for a further 5 minutes.
9. Stop the pump and place the retentate and input tubes into the 70% EtOH container. Start the pump again and let it run for 5 minutes. During this time, replace the water container with fresh distilled water.

Note: The fibers within the tube may start to distort, this is normal and the tubes will return to normal after the final water flushing stage.

10. Stop the pump and perform a final water flush for 10 minutes, 5 minutes with a pressure at 1 bar, 5 minutes with no pressure (by opening the flow control valve).
11. When the flushing has finished, remove the input tube from the container to allow air to pass through the system.
12. Continue to flush air through the system for 2 minutes.

F. Storage Protocol

Following the use of EVlution™, it is important to store the cartridge in a storage buffer, to both stop the membranes from drying out, as well as mitigate microbial fouling.

We recommend the use of 0.1M sodium hydroxide (NaOH) storage buffer, which is an effective sanitization agent for tangential flow filtration. This process is intended to prolong the storage of the EVlution cartridge. It is recommended that the storage buffer is replaced every 3 months when the cartridge is not used.

1. Ensure the cartridge is emptied of permeate following Operation protocol - section E *System cleaning* and flushing'. The cartridge should be emptied of all fluid.
2. Ensure the cartridge has the permeate outlets pointed upwards and that tube F remains attached to the permeate outlet.
3. Remove tube D from the outlet of the cartridge. Add a red rubber stopper to the outlet to stop the exit of fluid from the outlet.
4. Set the pump to 25 RPM.
5. Place tube A + F (input and permeate tubes) into the 0.1M NaOH solution.

6. Press ►/ ■■ on the pump and allow 15mL to pass through the pump, to fill the cartridge. Press ►/ ■■ again to stop once 15mL has passed through and entered the cartridge.
7. Take the cartridge out of the filter clips and carefully hold the cartridge vertically, to allow the 15mL of 0.1M NaOH to collect at the bottom of the cartridge (tube D side).
8. Push the 'DIR' button the pump. This will reverse the direction of flow, allowing the pump to drain any excess fluid currently still in tube C. Allow this to drain and empty past the 3-way stopcock connected to tube C.
9. Turn the 3-way stopcock connected to tube C 90° anticlockwise, to restrict any fluid from leaking out.
10. Whilst still vertical, remove tube C from the outlet of the cartridge. Apply a red rubber stopper to the outlet.
11. Place the cartridge back onto the filter clips, horizontally and with the outlets facing upwards.
12. Remove tube F and apply a red plug to the outlet. This will complete the addition of storage buffer to the cartridge.

The cartridge can be stored on the stand or in any other suitable location at room temperature.

EVlution Peristaltic Pump Specifications

Power supply	DC24V 1.9A power adapter
Power	Less than 48W
Motor type	Stepper motor
Motor life	5 seconds/time up to 4320000 times
Pump head	KAS
Working environment	Temperature 0°C~60°C, Relative Humidity < 80%RH
Storage environment	Temperature -20°C~80°C, relative humidity 10%~90% (non-condensing)
Total weight	510g
Reference noise value	≤47dB
Dosing accuracy	<±2%
Display method	Color LCD screen
Control method	Membrane buttons, speed control knob, foot pedal
Operating mode	Time mode, volume mode
Working mode	Continuous addition, cyclic addition
Support function	Start and stop, speed regulation, calibration
Tube code & size	B04 : 1.0x3.0, B06 : 2.0x4.0, B10 : 3.0x5.0
Tube material	Pharmed BPT
Max flow	B04 : ≥15ml/min, B06 : ≥42ml/min, B10 : ≥86ml/min

Troubleshooting

No liquid is flowing from the permeate output

- Possible cause:
There is not enough pressure to force the permeate out.

Ensure that all components are securely attached, and that the pressure syringe is at the 1 mL mark. If it is not, adjust the control valve.

- Possible cause:
Pores in the hollow fiber filter may be clogged

Repeat the cleaning step, but with a 1-hour wash cycle of NaOH, then flush through with 50°C distilled water for 10 minutes. Flush through with room temperature water for 5 minutes, then continue to the 70% ethanol.

Pressure is rising during filtration

- This may indicate that air is accumulating on the surface of the microfibrils. Try positioning the cartridge vertically. If the rise in pressure is very slow, this may indicate accumulation of debris from the input sample.

How do I remove bubbles from the final concentrated sample?

- The final retentate may have bubbles. Transfer the sample into an appropriate container and centrifuge. Centrifugation is dependent on the sample. For cell culture media, 1000 rpm for 5 minutes should suffice. Repeat as necessary.

How do I remove residual liquid from permeate tube

- Place the end of the tube into a waste container. Detach the permeate tube from the permeate outlet on the TFF. The liquid should flow into the waste container.

Can I process samples >1 L?

- We have not assessed this at present. Currently, we do not recommend using the 94cm² hollow fiber filter for input sample sizes that are greater than 1 litre due to potential clogging and fouling of the membrane.

Why is my concentrated yield lower than expected?

- Possible cause:
There is a low concentration of EVs in the starting material.

Quantify the particle concentration of your cell culture media (e.g. NTA) before using EVlution™.

- Possible cause:

The pore size of the hollow fibre filter is not optimal for the size range of the EVs within the sample.

Filters with various pore sizes can be purchased via www.cellgs.com.

See the table below:

Pore Size (kDa)	Approx. Pore Diameter (nm)	Retained Molecules	Typical Applications
5	~3	Small proteins, nucleic acids, EVs	Protein concentration; removal of salts and small molecules
8	~4	Proteins, nucleic acids, EVs	Protein purification; diafiltration
10	~5	Proteins, cytokines, nucleic acids, EVs	Cytokine and growth factor concentration; buffer exchange
30	~8	Large proteins, antibody fragments, EVs	Antibody fragment purification; depletion of small proteins
50	~12	Albumin, large proteins, EVs	Albumin removal; large protein concentration
100	~18	Large protein complexes, EVs	Concn. of protein complexes; pre-filtration for EV isolation
300	~25	EVs / Exosomes, large complexes	EV concn. and purification; FBS-EV depletion
500	~45	EVs / Exosomes, microvesicles	EV concn. and purification; FBS-EV depletion
750	~65	EVs, microvesicles, large particles	Microvesicle isolation; removal of smaller EVs and proteins
1000	~100	Large EVs, microvesicles, apoptotic bodies	Large particle concentration; apoptotic body isolation

Can I reduce the final concentration volume?

- It is possible to reduce the volume, but it should be taken into consideration that the void volume in hands-on mode is 5 mL. Reducing the total volume below 5 mL can create a lot of frothing/ bubbles which can make it difficult to determine the final volume.

Liquid is flowing through the system with permeate emerging but the pressure gauge isn't working.

- Possible cause:

The pump flow is in the wrong direction

Check that the pump flow direction is correct (anti-clockwise). If it is not, press the "DIR" button to reverse the flow.

- Possible cause:
Cartridge is leaky. See FAQ "how to know if the cartridge is compromised".

How to assess if the cartridge is compromised or

After prolonged or improper use, the cartridge may become clogged or develop holes in the hollow-fibres which allow the retentate to leak into the permeate.

- It is recommended to replace the cartridge after a maximum of 20 uses. The cartridge may need replacing sooner than this if the following is experienced:
 - The permeate flow is slower than usual
 - The permeate does not flow at all, even with the pressure set correctly. Do NOT attempt to increase the pressure above the recommended level as this may cause the tubing to disconnect.
 - The pressure gauge does not move despite turning the control valve completely closed and permeate emerging.

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