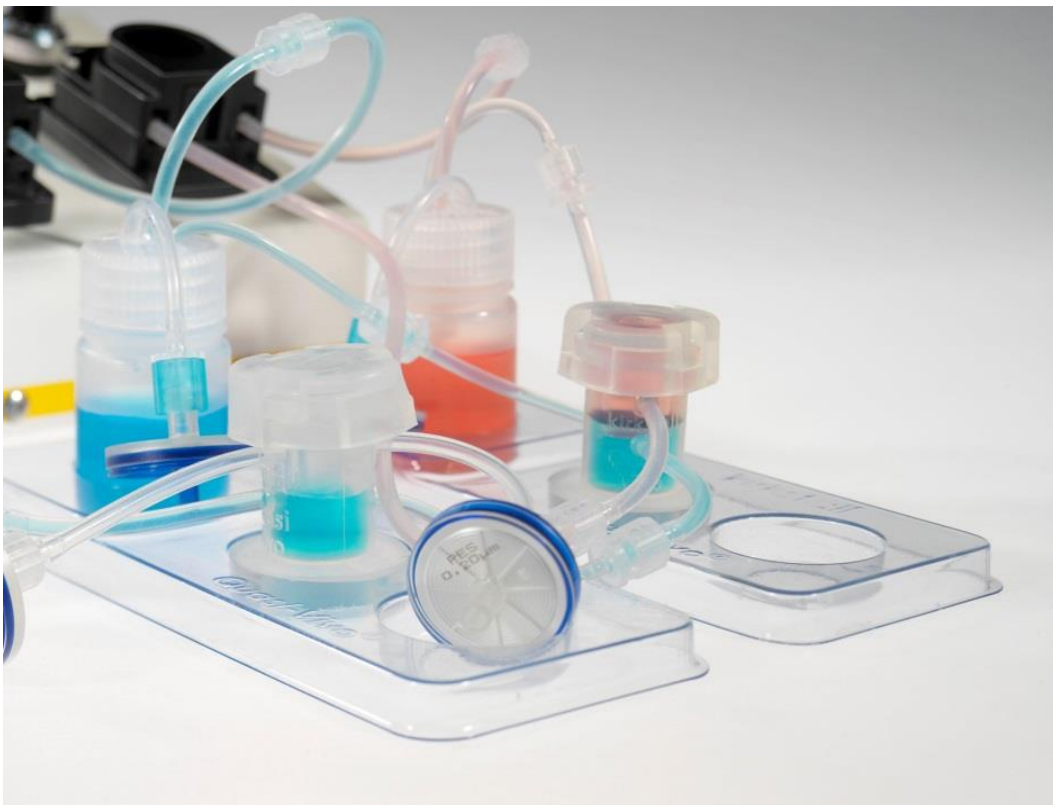
QV600



Tools for Physiologically Relevant *in vitro* Cell Culture

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Kirkstall | Lonza


Quasi Vivo system
kirkstall

CONTENTS

This User manual is specific to the Quasi Vivo® QV600 cell culture system for models of cells located on the interface of air and liquid. It is aimed at scientists to enable the transition from static culture to perfusion culture techniques. We advise that you read this document thoroughly before starting an experiment. The methods described may be adapted to suit specific applications.

For further support, training courses demonstrating the assembly and use of the Quasi Vivo® cell culture system are available and you can contact the technical support team:

US: scientific.support@lonza.com
EUROPE: scientific.support.EU@lonza.com

CONTENTS OF YOUR QV600 STARTER KIT

Your Quasi Vivo® system is delivered as a kit which includes:

- 3 silicone chambers with fitted connectors
- 1 reservoir bottle
- 6x 22 cm extension tubing in each of two diameters
- 6 each male and female luer connectors to fit each size of tubing
- A standard 0.2 µm filter
- 3 holding trays

- Spare tubing and connectors can be supplied, as well as additional items such as sampling ports; please contact scientific.support@lonza.com / scientific.support.EU@lonza.com

SETTING UP

Sterilisation

- Quasi Vivo® chambers are provided sterile. If chambers have been used, they can be autoclaved under standard conditions (121 °C at 15 psi for 15 minutes).
- Tubing, luer locks and the reservoir bottle should be autoclaved before first use (121 °C at 15 psi for 15 minutes).
- The filter is single use only, and should be replaced after each experiment.

How to connect the QV600

All steps should be done in a Class II biosafety hood, following standard sterile procedures.

1. Sterilise pump tubing (to be mounted on the pump rollers) by cleaning with 70% ethanol and then washing 3 times with PBS. Alternatively, this can be autoclaved.

> **NB** Check the pump tubing for wear and tear before and during every experiment. Due to the nature of peristaltic pumps, this tubing will degrade over time due to the action of the rollers. We recommend that you replace the tubing for the Parker pumps (PF22X0103 and PF600) after 20 days of continual use.

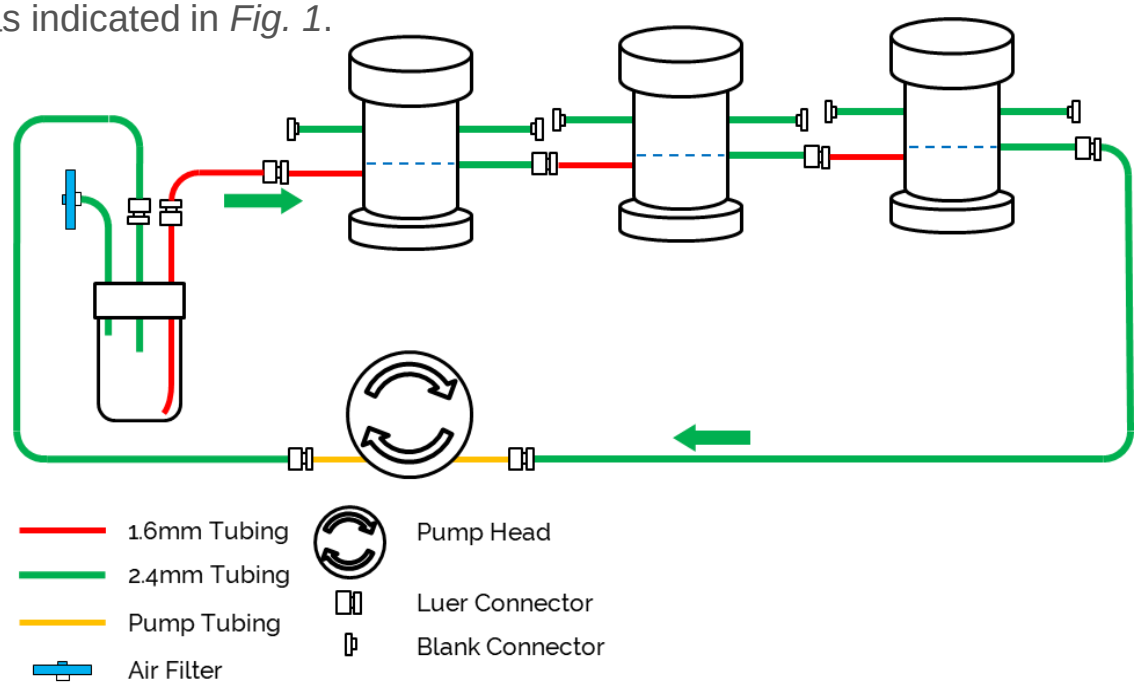
2. The reservoir bottle has tubes of three different lengths:

a) Short, with blue luer lock: the air filter should be attached here. This enables sterile gas exchange in the reservoir during the experiments and ensures that the internal and external pressures are equalized.

b) Medium: for the medium to return into the reservoir bottle. This is usually referred to as the return tubing.

c) Long: for medium to be pulled from the reservoir bottle, into the circuit. This is the pump outflow tube.

3. Construct your Quasi Vivo® circuit, working around the circuit from the pump tubing in the direction of flow using the correct sized tubing, as indicated in *Fig. 1*.



Some tubing will require the fitting of an appropriate luer lock; use the figure for guidance. Use the loading tray provided to hold the reservoir bottle and chambers in place.

a) Connect a length of 2.4mm tubing to the end of the pump tube; either using a luer connector or by attaching the tubing directly to the connector fitted to the pump tubing. Connect this tube to the return tubing of the reservoir bottle (the medium length tube).

b) Connect the outlet tubing of the reservoir bottle (the longest one) to the lower chamber inlet using a length of 1.6mm tubing fitted with luer connectors

c) Connect the lower chamber outlet to the pump inflow using a length of the 2.4mm tubing. It is recommended that you construct the loop with one chamber first, and then add in additional chambers once the circuit is complete.

It is helpful to remember “thin is in”; the smaller diameter tubing is always on the inlet side of the QV500 chamber.

4. Once your circuit is complete, add in any extra chambers needed, remembering to put them in the correct orientation (“thin is in”). Any extra tubing required due to experimental or space requirements can also be added in at this stage.

5. The upper chamber inlet and outlet can be connected to a gas supply if required, or otherwise can be sealed either with blanking plugs, air filters, or by connecting the two tubes together.

Pumps & Calibration

The Quasi Vivo® system uses a peristaltic pump to create flow. This pump design can generate low pressure, low velocity flow, which avoids causing stress or damage to the cells in the system. See below example flow rates.

Cell type	Flow rate (µl min^{-1})	Reference
Rat primary hepatocytes	180-500	Mazzei et al. 2010
Human primary hepatocytes	250-500	Vinci et al. 2011
hESC-derived hepatocytes	100-300	Rashidi et al. 2016
Oral and skin fibroblasts	75-150	Nithiananthan et al. 2016
Co-culture: Endothelial cells, hepatocytes and adipocytes	250	Vinci et al. 2012; Iori et al. 2012

Most such pumps have variable speed control, however the flow rate in the system will also vary depending on the number of chambers, the diameter and length of the tubing, the position of the chambers and reservoir in relation to the pump (for instance, placing on a higher shelf in the incubator) and, most importantly, on the type of pump used.

It is important to be aware of and control for these variables, which could influence your experiment, and so the system will need to be calibrated before use to ensure the flow rate is correct. Any subsequent modification to the system setup should be followed by recalibration.

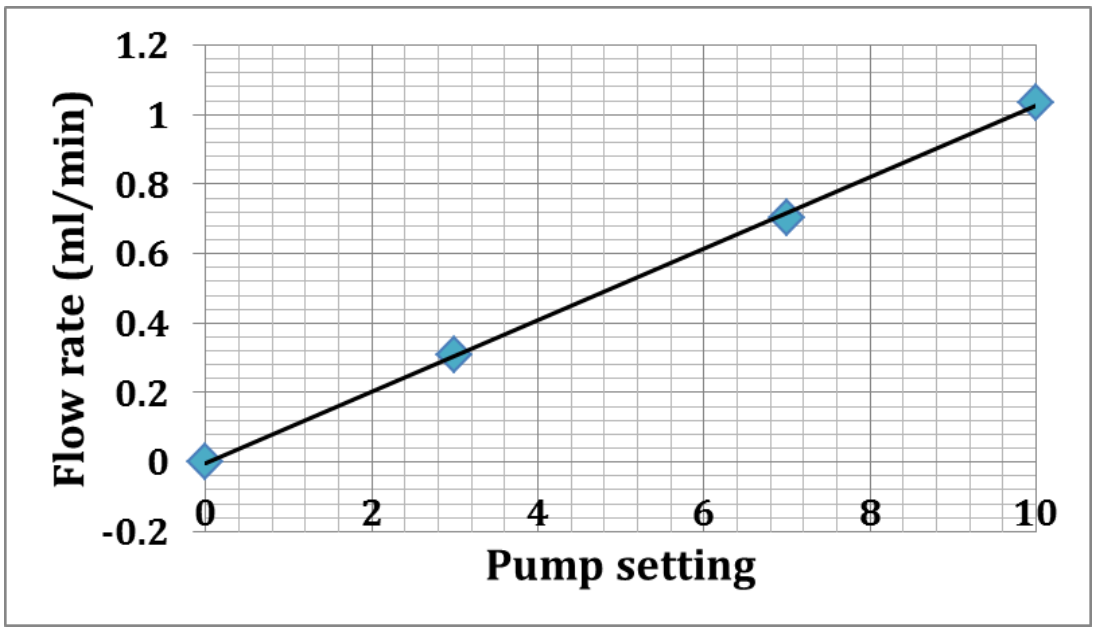
Depending on the pump you choose to use, the calibration process will vary. Kirkstall supply a low cost dual-head pump produced by Parker Hannifin (model PF22x0103) specifically for use with the Quasi Vivo® system, however any peristaltic pump capable of supplying the required flow rates can be used. If your pump does not provide you with a readout of the actual flow rate, you will need to do the following to translate the pump speed to flow rate.

This process is required for the Parker pump. To calibrate the system:

1. Run the required configuration filled with sterile PBS and collect the liquid output over 5 minutes from the final chamber. It is important that air is expelled from the system before starting to measure the flow rate.

2. Measure the volume of liquid circulated by weighing the medium in a weighing boat after 5 minutes- remember to weigh the boat empty first.

3. Do this procedure three times for each of three different settings (for example, low, medium and high speed), then plot the mean values on a calibration curve, an example of which is shown in *Fig. 2*.



Washing and Priming the QV600

1. If you have followed the calibration scenario described in the previous section in aseptic conditions, you will have already washed the system. If not, the connected system should be washed by filling the reservoir bottle with sterile PBS and connecting the closed system to the peristaltic pump at maximum flow rate for at least 20 minutes.

2. The PBS can then be removed from the reservoir bottle. The PBS in the chambers and tubes should also be removed; this can be achieved by lifting the chambers above the reservoir to allow the liquid to return to the reservoir. This emptying procedure should never be used when cells are in the system.

3. The system should be primed with the culture medium to be used in the experiment, in the same manner as the PBS wash, and this medium then removed. The system is now ready to be used in an experiment.

Starting your experiment

1. Prepare cells as per normal experimental requirements, and seed onto hanging inserts with the required membrane properties. We recommend that cells are maintained under static conditions for some time before being transferred to the chambers, to allow cells to adhere to the transwell or insert. The time is dependent on cell type and experimental conditions - talk to one of our technical team for guidance.

2. Open up each chamber and add the inserts.

3. Pipette 1.5 ml of cell culture medium into each chamber. This reduces the filling time when starting the system. The media level should not reach the chamber outlet, as this may affect the height of the media after filling the chamber.

4. Refit and seal the chamber lids, and ensure the circuit is fully sealed and that the connector tubing has no kinks or obstacles. It is a good idea to check that each luer lock has been secured tightly.

5. Add the required amount of medium to the reservoir bottle, taking into account the volume already added to each chamber.

6. Transfer the set-up to your incubator. Connect the pump tubing to the pump, set your pump speed and switch the pump on. Check the system after 10 minutes to ensure that fill up is complete.

7. If desired, control samples of cells can be kept in static conditions, either in static QV600 chambers (with the tubing looped back to itself) or in a standard cell culture plate.

DURING YOUR EXPERIMENT

Analysis

The QV600 chambers are compatible with a number of analysis techniques, including (but not limited to) Western Blotting, RT-PCR, microarray analyses, immunohistochemistry and viability assays such as the MTT assay. The simplest way to perform these assays is to remove your cells from the chamber and process them in the same way as you would for static culture. However, there is an increasing need for inline measurements, and these can be done in the QV600 by introducing sampling ports into the circuit; these contain a rubber septum through which a needle can be pushed to withdraw medium for analysis or inject into the system. Please contact the Kirkstall technical team for more information.

Medium change

A change of the medium is generally necessary every 3-7 days. This depends on how many chambers you have connected to one reservoir bottle, the volume of medium in the reservoir bottle, and how metabolically active your cells are. We recommend that you replace half of the volume of your system during each change rather than the full amount, to ensure that conditioned medium is maintained.

Pump Maintenance

When using a peristaltic pump, the pump tubing is exposed to wear from the pump head. Check with the manufacturer of the pump tubing regarding how long you can run the pump and at what speed before the pump tubing starts to deteriorate. With the Parker pump (PF22X0103), the pump tubing should be changed after 20 days continuous use.

You should inspect the pump tubing and system when you perform medium change for signs of wear, such as the following:reduced transparency in the tubing, a rougher feel to the exterior of the tubing, cracking within the tubing, increased number of bubbles in the system.

ENDING YOUR EXPERIMENT

Disassembling the QV600

1. Stop the pump and detach the system by removing pump tubing from the rollers.
3. Move the system into the hood.
4. Detach the reservoir bottle from the circuit, but maintain sterility by connecting the two Luer locks together, creating a circuit without a reservoir.
5. Open the chamber lid.
6. Remove the insert from the chamber using forceps and place them into your chosen assay receptacle, eg a standard cell culture plate.
7. Follow the manufacturer's instructions for your chosen assay. For example, this could be an MTT assay or immunohistochemical staining; remember to perform the same assay for your static controls.

Cleaning the QV600

Procedure A: The chambers can be used for more than one experiment without autoclaving, if the rules of aseptic techniques to prevent contamination are strictly followed.

1. Circulate 70% ethanol through the whole system for 1-2 hours, by connecting the whole system together and adding ethanol to the reservoir bottle, then switching on the pump.
2. Rinse VERY thoroughly with sterile PBS (ideally overnight) to remove any traces of ethanol which might affect cell viability.

3. Tubing, connectors, chambers and reservoir bottles can be stored after opening for longer periods of time. In this case, tubing and connectors should be stored submerged in 70% ethanol.

4. Chambers and reservoir bottles should be rinsed with 70% ethanol followed by sterile PBS (long exposure to ethanol can damage the surface of silicone chambers), emptied and stored until needed. Used air filters on reservoir bottles should be discarded.

Procedure B: Silicone parts, as well as reservoir bottles, can be sterilised using standard autoclaving procedure (121^{o}C, 15 psi, 15 minutes).

However, to maintain reliability of the system we advise not to exceed more than 3 sterilisation cycles for any component. The translucent polypropylene luer lock connectors supplied with the kit are also autoclave safe.

We advise NOT TO wash with sodium aside which can accumulate on the surfaces of chambers and leak out during the experiment.

FAQs

1. **Why is the system leaking?**

This should not happen in normal operation. Check all the connections between tubes and ensure the chambers are correctly assembled. Check the tubing for breaks due to fatigue. If the chambers have been autoclaved more than 3 times, the leakage could be due to changes in the silicone.

2. **Can you reuse the system?**

QV600 chambers can be autoclaved up to 3 times. The reservoir bottle, tubing and luer lock components can be autoclaved repeatedly.

3. **Is the medium recirculating or single pass?**

The system is designed to use recirculating medium, which allows cells to condition the medium with growth factors and signaling molecules, and therefore improves growth, viability, and the system's ability to model the in vivo environment. However the system can easily be set up to allow single pass where required.

4. **How many chambers can be connected to the same reservoir bottle?**

In a recirculating system, up to 6 chambers can be connected to a single 30 ml reservoir bottle. Larger or smaller bottles will support different numbers of chambers.

5. **How long can cells be cultured for?**

Hepatocytes have been cultured for a month and have retained their phenotype and CYP gene expression. The length of the experiment can be varied from 1 to 3 day experiments, to week or month-long studies and potentially longer.

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