OPERATING INSTRUCTIONS

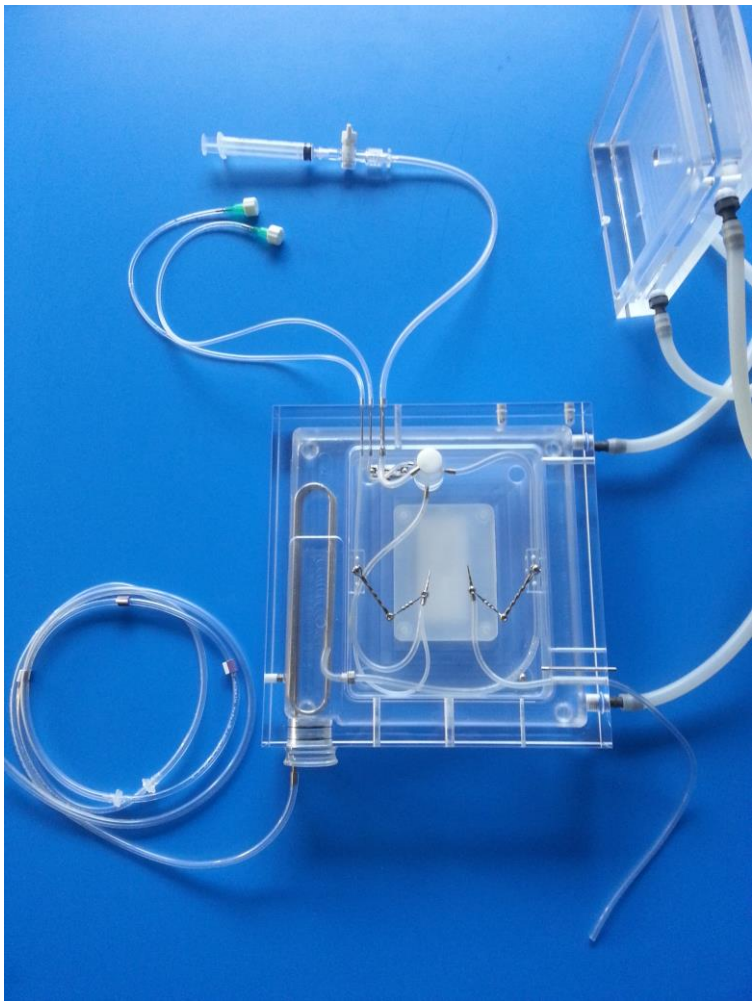
for the

HSE TEMPERATURED MOIST CHAMBER (73-2901)

Type 834/8 with metal tube heat exchanger

for ex-vivo abdominal organ perfusion of small rodents

(Version 2.4 / TB / Sep. 2025)



NOT FOR HUMAN USE

Contence

1. Introduction, manufacturer's details

These operating instructions describe the function of the HSE Temperature Controlled Moist Chamber Type 834/8 for ex-vivo abdominal organ perfusion from small rodents. They are a part of the equipment and should be kept closed to it.
All the information in these instructions have been drawn up after careful examination but do not represent a warranty of product properties. Alterations in line with technical progress are reserved.

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2. Safety, hazard notes, and reservations

Several references are made in the text of these Instructions to hazards which might arise during the use of the product described. Special attention is drawn to the fact that this cannot exclude other hazards not specifically mentioned here. It is the responsibility of the user himself to judge his actual experimental setup - in conjunction with the auxiliary and test substances used - in respect of possible hazards. In case of doubt, he is responsible for obtaining suitable advice from a fully experienced person.

Appropriate safety precautions must be observed during the setting up, installation and operation of the apparatus/setup. A few points are listed below which should be observed to prevent or avoid any danger to the user; they do not claim to be comprehensive.

- Electrical equipment is generally designed to Class 1 protection (metal housing connected to the ground contact of the mains supply). It must only be connected to correctly installed wall outlets with ground contact. In case of doubt, ask a qualified electrician! Damaged socket outlets should be repaired immediately and must not be used. Always adhere to these precautions.

- Use only perfect, undamaged and dry mains power cables.

- Protect electrical equipment against moisture:
  - o Never place electrical equipment underneath stored liquids!
  - o Do not position electrical equipment near a water tap where it may be splashed!
  - o Electrical equipment should only be operated with dry hands!

- If any liquid has found its way into the equipment or even if this is only suspected, do not switch on the equipment. Immediately pull out the mains plug and have the equipment checked by an electrician. Remember; if you notice any smoke or smell it will be too late!

- When using toxic substances or gases, appropriate precautions must be taken in order to avoid any danger to the operator or contamination of the working area. Remember: smell is no indication of toxicity, and conversely: a toxic substance need not smell!

- Remember the fire hazard! Combustible substances in contact with Carbogen gas (= 95% oxygen) represent a fire hazard. Observe the appropriate guidelines and regulations!

3. Introduction - general description of the chamber

The Temperature Controlled Moist Chamber Type 834/8 with metal tube heat exchanger is intended for use in ex-vivo perfusion studies on abdominal isolated organs of small experimental animals, such as kidney, liver, spleen or mesenteric bed of mice, rats or guinea pigs. Also hearts can be perfused in horizontal position e.g. for imaging. The chamber has been designed to perfuse the above-mentioned isolated organs under best physiological temperature conditions.
The organs in the chamber can be perfused under constant pressure or constant flow.
The temperatured Moist Chamber Type 834/8 can be used in combination with HSE perfusion equipment (pumps, reservoirs, oxygenators, filters etc.) and the HSE PLUGSYS measurement system with the SCP pressure regulator to provide constant pressure perfusion and calculate the flowrate of the organs.

4. Additional items required

In order to work with the moist chamber, it is necessary to have the following additional items:

- A thermocirculator, capacity approx. 3 - 5 litre, distilled water and algal growth inhibitor, e.g. Thermoclean DC.
- A Carbogen source for aeration. Either a central supply or a Carbogen cylinder with a pressure regulator. If additional gas mixtures are to be used, the appropriate connections or cylinders have to be provided.
- A reservoir for the perfusate, with a gas frit for aeration. The quantity of perfusate depends on the requirement of the intended experiment.
- In case of foaming perfusion hollow fiber oxygenators can be used.
- A pump, usually a peristaltic pump (for blood we also can offer small centrifugal pumps), with the required output, adjustable in speed up to 30 ml/min.
- An adapted cannulae set

Additionally we recommend for the accurate measurement and control of the perfusion pressure:

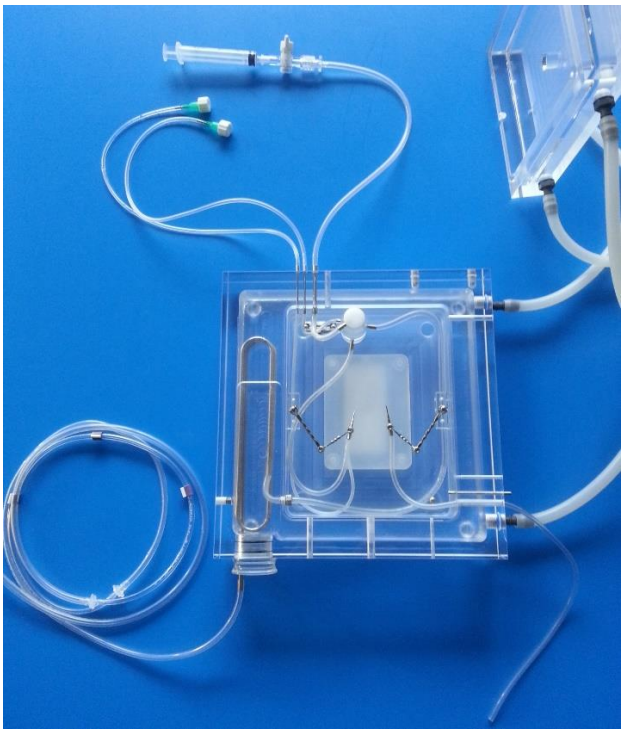
- A pressure transducer for measuring the perfusion pressure, e.g. an APT-300 or P75 transducer

- A bridge amplifier (e.g. HSE TAM-A or TAM-D) to amplify the signal and to make it suitable for a computerised signal data acquisition and evaluation system (e.g. HSE BDAS Software).

- A TAM-D Transducer Amplifier Module and an SCP controller module to perform constant flow or constant pressure perfusion with calculating the flowrate in ml/min from the pump speed.

Measurement of additional parameters (e.g. perfusion flow measured with an ultrasound transit time flowmeter) may be useful depending on the particular application involved in the experiment. The necessary measuring equipment must then be added to the complete apparatus. In general all is modular and a system can grow with the application and needs.

5. Detailed description



The moist chamber consists of the bottom (the actual chamber) and the removeable cover. Both bottom and cover are jacketed and thermostated with warm water passing through them. Operation of the moist chamber therefore requires a laboratory thermostat with built-in pump.
The moist chamber is equipped with a metal heat exchanger to warm the perfusate and compensate the heart loss between reservoir and organ. The perfusate is warmed in the heat exchanger and then passes directly into the interior of the chamber. Thus, it cannot cool down. Therefore, the perfusate temperature corresponds exactly to the water temperature.

To enable the fixation of organs a silicone pad is fitted onto the bottom of the interior chamber. The silicone pad can be removed e.g. for cleaning the chamber. Inside the moist chamber there are two Plexiglass strips on both sides, each with four threaded holes (M2). These holes are provided so that 5 mm dia. balls can be screwed into them. The balls take the clip-on segments of the mini ball joint holders. The mini ball joint holders are used to ensure that the connecting cannulas (also fitted with suitable balls) for the perfused organ are held in the required position. They can also secure other devices such as electrodes, tubing or catheters.

A number of apertures and stainless steel tubes are provided in the chamber walls; they can be used e. g. for:

- Supply and discharge of perfusate
- Connection of measuring probes (e. g. pressure transducer)
- Discharge of bile (liver perfusion)
- Discharge of urine (kidney perfusion)
- Infusion of drug

Under **constant flow condition** the moist chamber is used together with a roller pump supplying the required flow rate. The perfusate equilibrated with gas (O2 / CO2) is drawn by the pump from a reservoir. If **constant pressure perfusion** is required, the pressure will be measured with an APT-300 or a P75 pressure transducer, connected to a TAM-D module and an SCP controller module. The SCP controller will regulate the pump speed with a build in PID regulator to keep the pressure constant. On the SCP module you can set the wished pressure (SET POINT). As the SCP knows the pump speed it also calculates the flow in ml/min. (More see chapter 7).

Figure 1 shows an overview of the moist chamber.

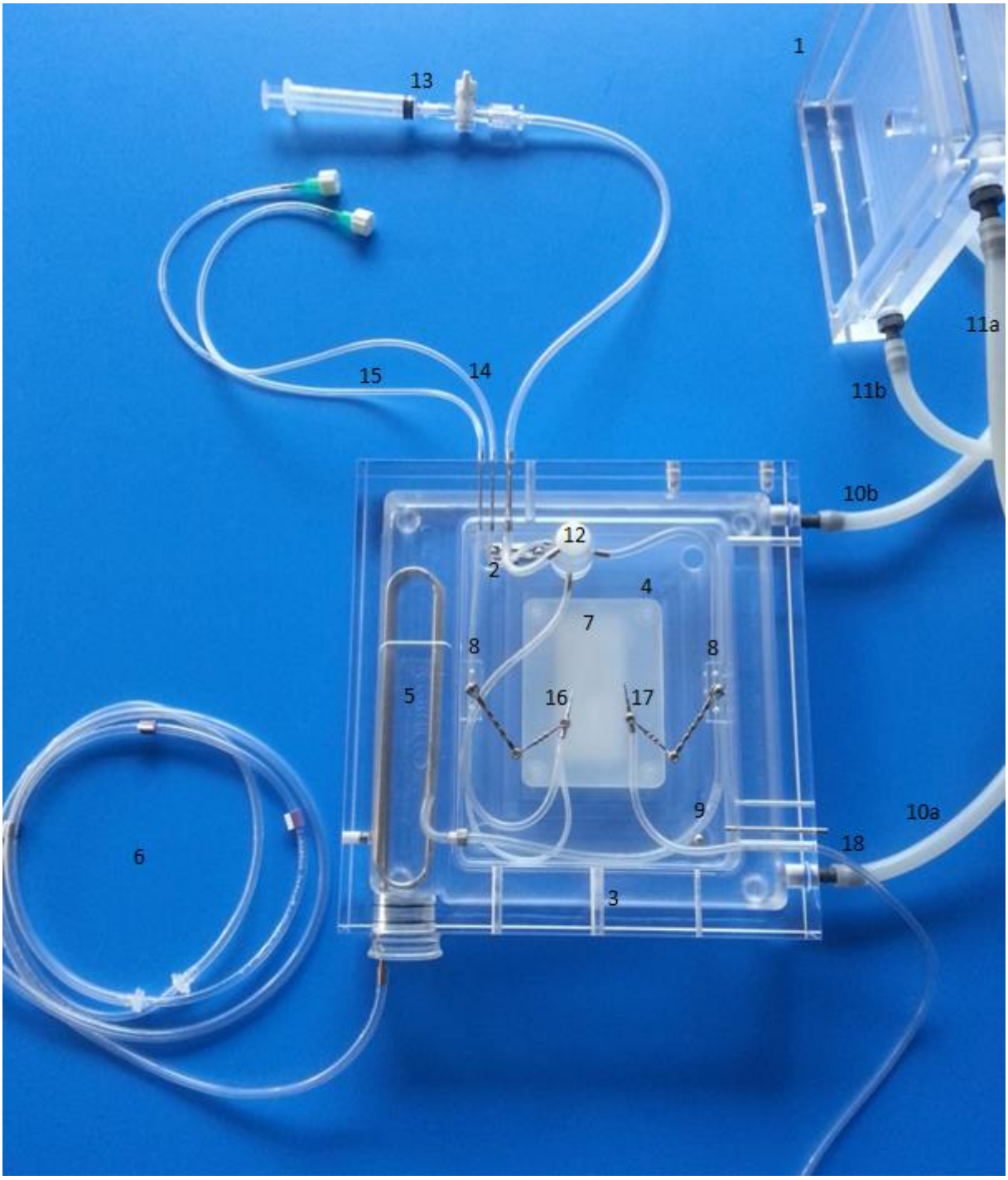


Fig. 1: Moist chamber, seen from above with cover removed.

1 Jacketed lid of the moist chamber.

2 Holder for bubble trap (option). The bubble trap is plugged onto this holder.

3 Ports through the walls of the moist chamber, for use as required (e.g. for connecting a pressure transducer APT-300 or P75).

4 Bottom of the moist chamber. The bottom is jacketed. The inner of the chamber has to be equipped with cannulae to perfuse the isolated organ. To avoid dehydration of the isolated organ the bottom of the moist chamber has to be filled with a small amount of perfusion solution.

5 Heat exchanger of the moist chamber. Warm water from the thermostating circuit passes around the stainless-steel tube. The perfusate is pumped through this tube and effectively

	warmed. The passage through the chamber must be water-tight to ensure that no water from the thermostatic circuit passes into the chamber.
6	Tubing for perfusate supply from reservoir via roller pump to heat exchanger.
7	Silicone pad on which the organ is mounted. Organ can be fixed in position by inserting needles. The silicone panel is removable e.g. for cleaning the chamber.
8	Perspex strip with four threaded holes (M2) for securing the mini joint ball holders.
9	Tubing from heat exchanger (5) to bubble trap (73-3692) or cannula.
10a, 11a	Tubing from thermocirculator.
10b, 11b	Tubing to the thermocirculator.
12	Bubble trap
13	3ml syringe with stopcock to adjust air cushion in bubble trap
14	tube for drug addition
15	tube to measure perfusion pressure in arterial cannula
16	arterial cannula
17	venous cannula
18	venous outlet

6. Notes on using the moist chamber

6.1 General notes

The organ to be perfused ex-vivo is placed into the chamber at a suitable position on the silicone plate and if necessary fixed with needles. In general, a gaze layer can be is placed on the silicone plate or the silicone pad can be completely replaced by a gaze cushion.

Thermostating:
Connect the inlet and the outlet port of the cover and the bottom chamber to a thermocirculator. Ensure that bottom and cover are filled with warm water (37°C) bubble free.

Organ connection:
Immediately before mounting the organ ensure that the entire perfusion system up to the distal end of the perfusion cannula is filled with fresh perfusate and free from air bubbles. Arrange the organ connection canulae so that the vessels are not kinked and the perfusate can flow easily. The mini ball joint holders with the cannula are useful here.
The canulae are equipped with a basket like tip which allows easy insertion into the vessels and avoids any occlusion of the vessel during preparation and experiment.

Perfusion pressure:
During perfusion ensure that the pressure acting at the organ does not exceed the physiological perfusion pressure specific for this organ. This is only possible by using a suitable pressure measuring system (e.g. pressure transducer APT-300 or P75 with HSE amplifier module TAM-A or TAM-D). In order that the true pressure is being measured it is necessary to connect the pressure transducer as close as possible to the organ. To measure the pressures best is to use cannulae with side port.

Chamber environment:
After the chamber has been closed with the cover it takes a few minutes until the desired temperature and humidity are reached in the chamber. In order to saturate the air in the chamber with water vapour it is necessary to have sufficient water in the chamber. Always ensure that a little water or perfusate is on the bottom of the chamber. In an organ the surface of which has previously been moistened, dries quickly inside the closed chamber, this is a sure sign that there isn`t any liquid inside the chamber.
If possible do not open the chamber during operation. After it has been closed it takes some time for the warm, moist environment to become established again.

Bubble trap:
Watch the filling level of the bubble trap! Always fill it before starting the experiment to around ¾ with fluid.

Recirculating operation:
When operating with recirculation it is possible that tissue particles flushed out of the organ can reach the perfusate. In order to avoid micro embolism in the organ during perfusion it may be necessary to insert a particulate filter of 40 to 80 µm into the perfusion circuit, e.g. before the heat exchanger.
Always install the filter after the pump, never on the suction side.

6.2 Cannulating isolated organs

6.2.1 Mini ball joint holders and cannulas for organ connection

Inside the moist chamber are plexiglass strips on both sides, each with four threaded holes (M2). These holes are provided so that 5 mm balls can be screwed into them. The balls take the clip-on segments of the mini ball joint holders. The ball joint holders are used to ensure that the connecting cannulas (also fitted with suitable balls) for the perfused organ are held in the required position.

Mini ball joint holders and cannulas can be ordered separately (chapters 6.2.2 and 6.2.3)

6.2.2 Mini ball joint holders

The mini ball joint holders consist of arms of different lengths which carry a ball or a wire clip (eye) on the ends. Several arms can be clipped together according to the application. The ball joint holder allows the holder to be moved into any configuration. All holder elements are made from stainless steel.

Mini ball joint holders can be used e.g. for mounting of cannulas on small isolated organs like liver or kidney.

73-0176 Mini Ball Joint Holder, Eye-Ball, L=18mm
73-0177 Mini Ball Joint Holder, Eye-Ball, L=23mm
73-3321 Mini Ball Joint Holder, Eye-Ball, L=35mm

73-0174 Mini Ball Joint Holder, Eye-Eye, L=23mm
73-0175 Mini Ball Joint Holder, Eye-Eye, L=42mm

73-0563 Mini Ball Joint Holder, Ball-Ball, L=18mm

73-0564 Link for Higher Loading Capacity for Two Arms with Ball, Clamping Force Adjustable, L=23mm

73-0565 Universal Holder with Eye and Free End for Fixation ECG Electrodes etc., L=20mm

73-0178 Mini Ball with Thread

6.2.3 Cannulas for perfusion of isolated organs (e.g. liver, kidney)

The cannulas are equipped with a basket at the front to avoid any occlusion during preparation and experiment. They are available in different sizes depending on the species and with or without side port for pressure measurement or bolus injection.

The cannulas can be combined with mini ball joint holders.

73-3308	Cannula with Basket, OD 1.0mm
73-3310	Cannula with Basket, OD 1.3mm
73-3312	Cannula with Basket, OD 2.0mm
73-3314	Cannula with Basket, OD 2.3mm

73-3309	Cannula with Basket and Side Port, OD 1.0mm
73-3311	Cannula with Basket and Side Port, OD 1.3mm
73-3313	Cannula with Basket and Side Port, OD 2.0mm
73-3315	Cannula with Basket and Side Port, OD 2.3mm

Fig. 3: Cannulas for perfusing isolated organs.

6.3 Bubble trap (option)

An optional a bubble trap (catalog no. 73-3692) is available. It comes with a syringe to remove air bubbles and to adjust the air volume in the bubble trap. Tubing for connecting the syringe to the bubble trap are also included.

The bubble trap is installed in front of the organ to be perfused by fixing it on the holder in the interior of the moist chamber. It has a port to remove air bubbles using a syringe and a port to measure the perfusion pressure in front of the organ.

The bubble trap is suitable for flow rates up to approx. 50ml/min.

Fig. 4: Bubble trap for the moist chamber.

1 Port to adjust air volume and to remove air bubbles (OD = 2.5mm) using a syringe.
2 Port to measure perfusion pressure in front of the organ (OD = 1.3 mm) in case no canulae with side port is used. Can also be used to add drugs.
3 Ball with thread for fixing mini ball joint holders (e.g. Link for Higher Loading 73-0564).
4 Bore for fixing the bubble trap in the moist chamber (only on older chambers).
5 Output port to the organ (OD = 2.3 mm).
6 Input port from roller pump (OD = 2.5 mm).

OD = outer diameter

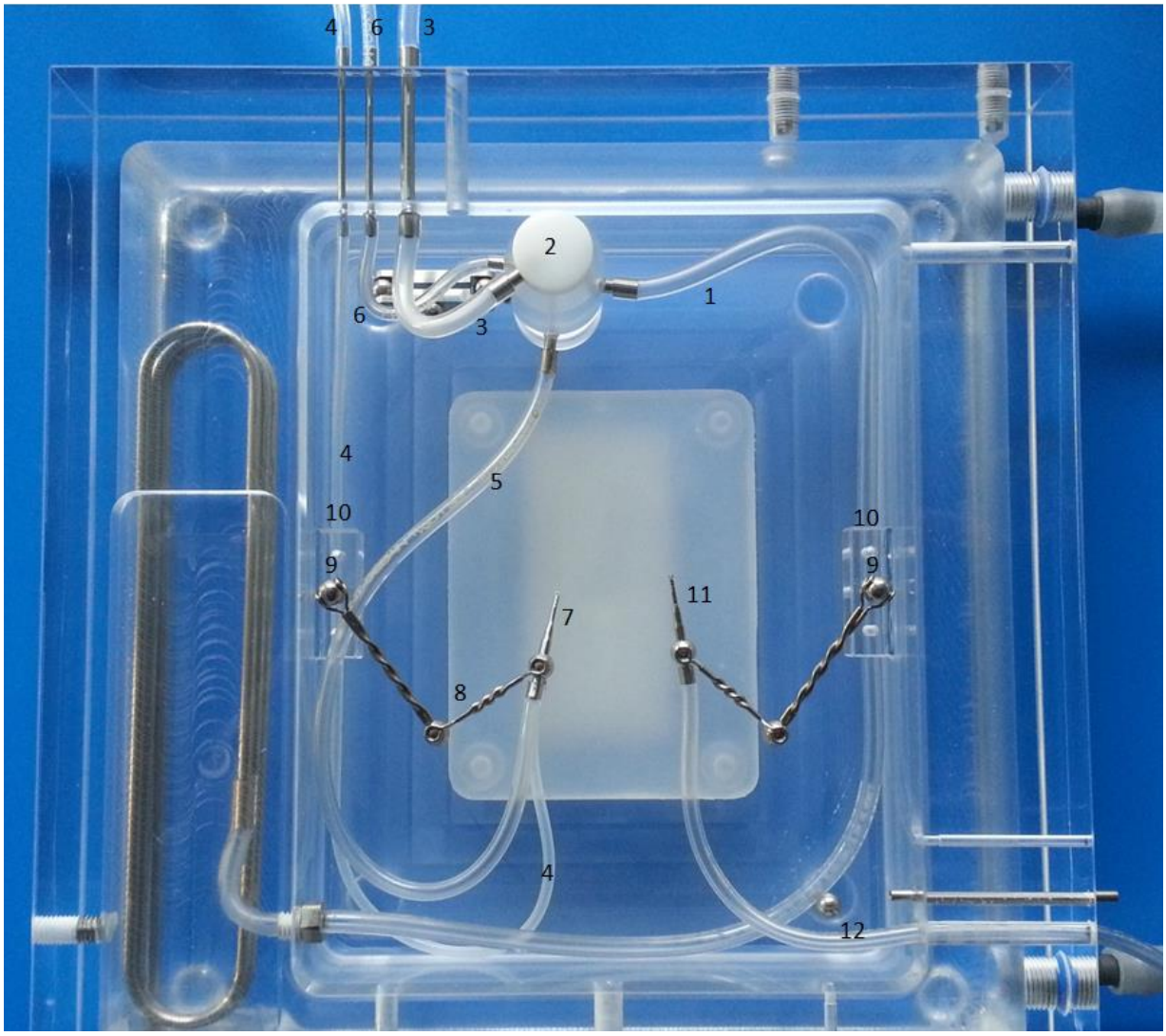


Fig. 5: Interior of the moist chamber with bubble trap, mini ball joint holders and cannulas.

1 Tubing from the heat exchanger to the bubble trap (2)
2 Bubble trap in front of the organ. The bubble trap has a port to remove accumulated air (3) and a port to measure the perfusion pressure or add drugs (6).
3 Tubing to syringe (not shown) to remove air bubbles or adjust air volume inside the bubble trap.
4 Tubing to measure perfusion pressure in front of the organ inside the arterial cannula
5 Tubing from bubble trap (2) to the arterial cannula (7) for perfusing the isolated organ.
6 Tubing e.g. for bolus injection into bubble trap. Can also be used for pressure measurement when tubing 4 is used for bolus injection.
7 Cannula with side port (arterial) for perfusing the isolated organ.
8 Mini ball joint holders for fixing the cannula (7).
9 Ball with thread (M2) to take mini ball joint holders (8).
10 Strip with three threaded holes (M2) for securing the mini joint ball holders.
11 Cannula (venous) for getting the effluent of the isolated organ.
12 Tubing from the venous cannula (11) to the waste or the perfusate reservoir in recirculating mode.

Avoid too long tubing, keep the tubing outlet on the level of the organ to avoid unphysiological afterloads which will result in oedemas. Never go with tubing 12 back into a higher elevated reservoir, the backpressure will be too high. The venous pressure in-vivo is only 2 - 4 mmHg. Not more than 5cm H2OIn case of recirculation let the venous outflow drip into a small glass beaker and use a second pump channel of your pump to pump the fluid back to the reservoir. Another option would be to lift up the whole chamber and put it on a small table e.g. our table 73-4198. In this case the outflow could go directly into the reservoir.

7. Examples of applications

7.1 Perfusion of the isolated liver or kidney

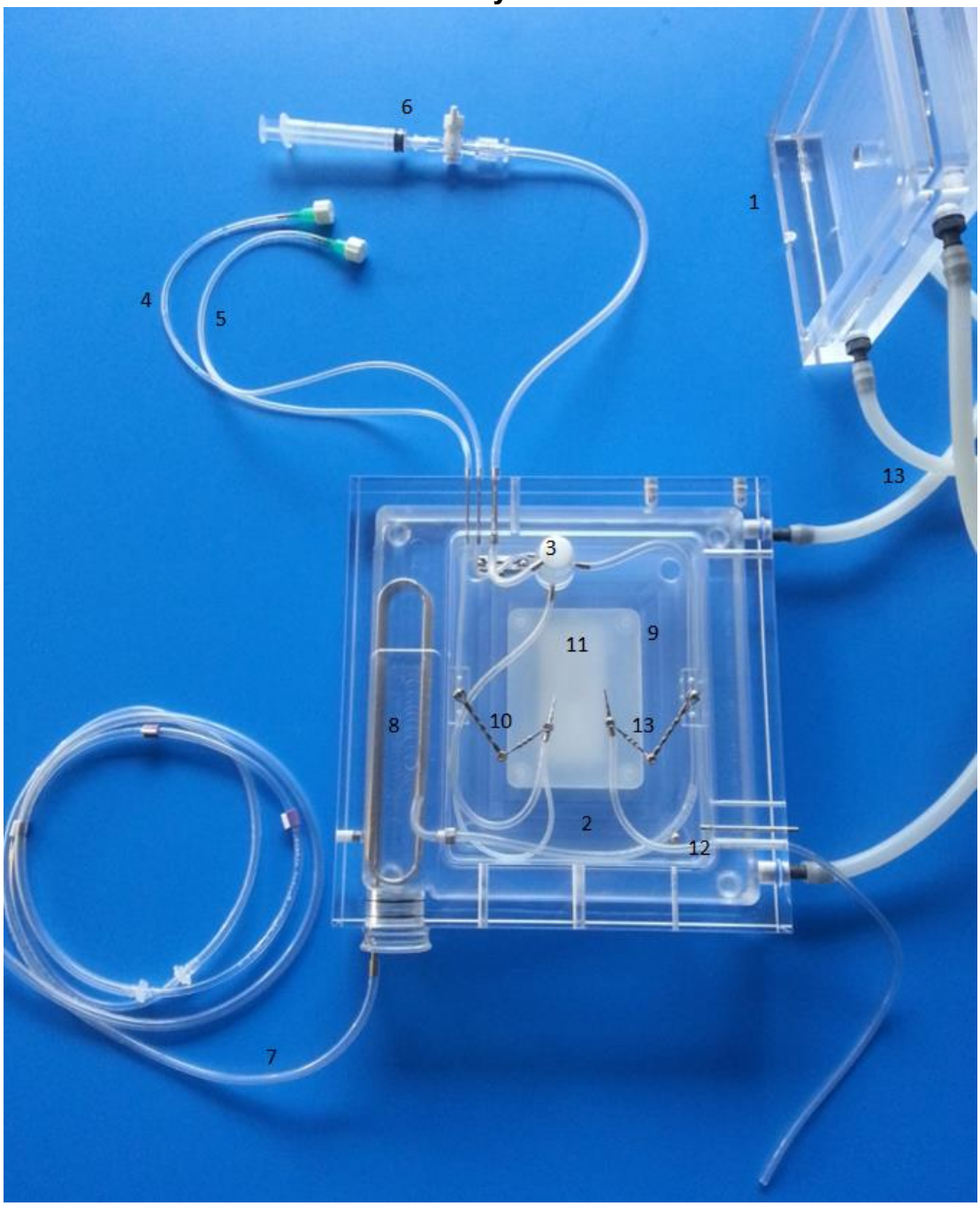


Fig. 6: Moist chamber equipped with cannulae for perfusing isolated organs as kidney or liver.

1 Cover of the moist chamber. The cover is jacketed.
2 Bottom of the moist chamber. The bottom is jacketed. Several tubes and apertures in the wall are used for cannulating the organ or for connecting measuring instruments required e.g. APT300 or P75 pressure transducer. The cover is removed.
3 Bubble trap in front of the organ. The bubble trap has a port to remove air bubbles and a port to measure the perfusion pressure.
4 Port for measuring the perfusion pressure in front of the organ. A pressure transducer (e.g. APT300 or P75) can be connected to this port.
5 Port e.g. for bolus injection into the bubble trap. Also 4 could be used. In this case 5 must be used to measure the perfusion pressure
6 Syringe with attached stopcock to remove air bubbles or adjust air volume inside the bubble trap (3).

7 Tubing for perfusate from reservoir via roller pump (not shown) to heat exchanger (8).

8 Heat exchanger of the moist chamber. Warm water from the thermostating circuit passes around this stainless-steel tube. The perfusate is pumped through this tube and effectively warmed. The passage through the chamber compensates the heat loss between reservoir and organ.

9 Inner of the moist chamber. At the bottom of the interior there is a removable silicone plate to carry the organ. The interior has threaded holes at the sides for fixing mini ball jont holders (10, 13) for supporting connecting canulae.

10 Mini ball joint holders and arterial cannula for perfusing the organ.

11 Removeable silicone plate to fix the organ with needles.

12 Tubing from the venous cannula (13) to waste or to the reservoir in recirculating mode. Avoid too long tubing, keep the tubing outlet on the level of the organ to avoid unphysiological afterloads which will result in edemas. Never go with tubing 12 back into a higher elevated reservoir, the backpressure will be too high. The venous pressure in-vivo is only 2 - 4 mmHg. Not more than 5cm H2O. In case of recirculation let the venous outflow drip into a small glass beaker and use a second pump channel of your pump to pump the fluid back to the reservoir. Another option would be to lift up the whole chamber and put it on a small table e.g. our table 73-4198. In this case the outflow could go directly into the reservoir

13 Mini ball joint holders and venous cannula for perfusing the organ.

14 Thermostat tubes for heating the bottom and cover of the moist chamber. Both, bottom and cover have an inlet and an outlet port for connecting these tubing.

7.2 Perfusion and stimulation of the isolated mesenteric bed

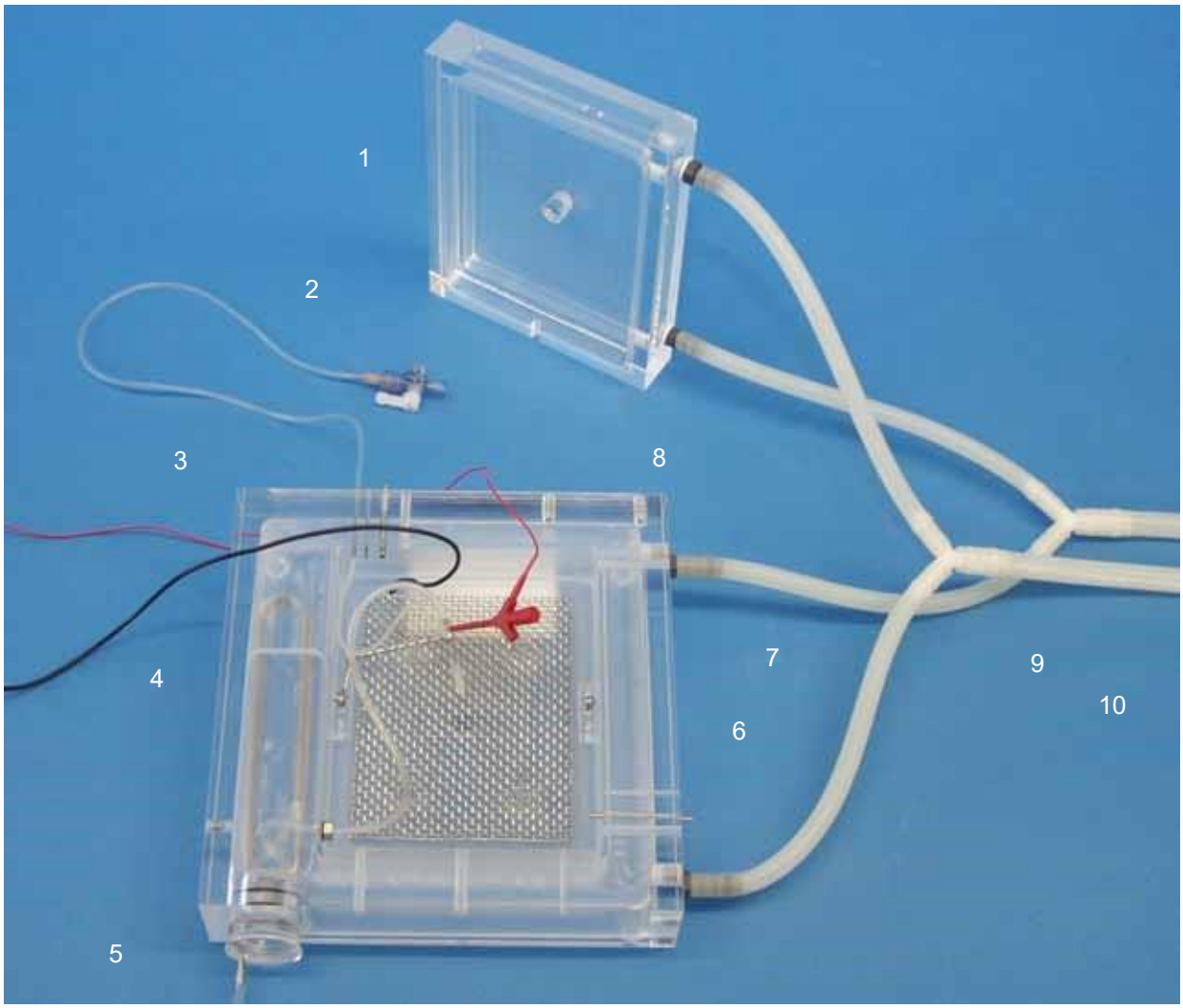


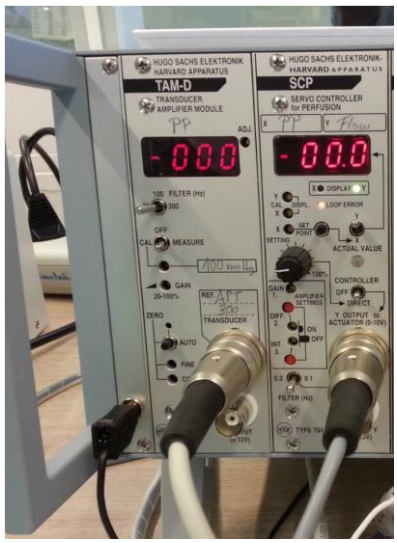
Fig. 7: Moist chamber equipped for experiments on a mesenteric bed.

1 Cover of the moist chamber. The cover is jacketed.
2 Tubing to introduce test drug through a roller pump or a syringe pump.
3 Bottom of the moist chamber. The bottom is thermostated.
4 Cable to anode for electrical stimulation (stainless steel mesh, 6).
5 Heat exchanger. Warm water from the thermostating circuit passes around the stainless steel tube. The perfusate is pumped through this tube and effectively warmed.
6 Stainless steel mesh. The mesenteric tissue is placed into the moist chamber on this mesh. The mesh also acts as anode during electrical stimulation.
7 Steel cannula for cannulating the superior mesenteric artery. The perfusion pressure is measured in the branch in front of this cannula.
8 Cathode. For electrical stimulation a metal clip as cathode (-) is attached to the aorta section near the steel canula.
9,10 Tubings to / from thermocirculator.

7.3 Moist chamber used together with the SCP Servo Controller for pressure and flow control

In most applications the moist chamber is used together with the HSE PLUGSYS pressure and flow regulation system. To perform constant flow or constant pressure perfusions two modules in a housing will be required:

- a TAM-D which measures and displays the pressure in mmHg
- a SCP module which controls the pump and calculates and displays the flow in ml/min.



Example 1: TAM-D and SCP installed in a PLUGSYS housing Type 601

Example 2: TAM-D and SCP installed in a MiniCase housing Type 609 together with an USB-C Control module to record pressure and flow with a Data Acquisition System (DAQ),

A system requires a HSE TAM-D with an APT-300 pressure transducer and a SCP module together with a suitable peristatic pump with an analog voltage input.

On the next page you will see a scheme of a servo controlled system

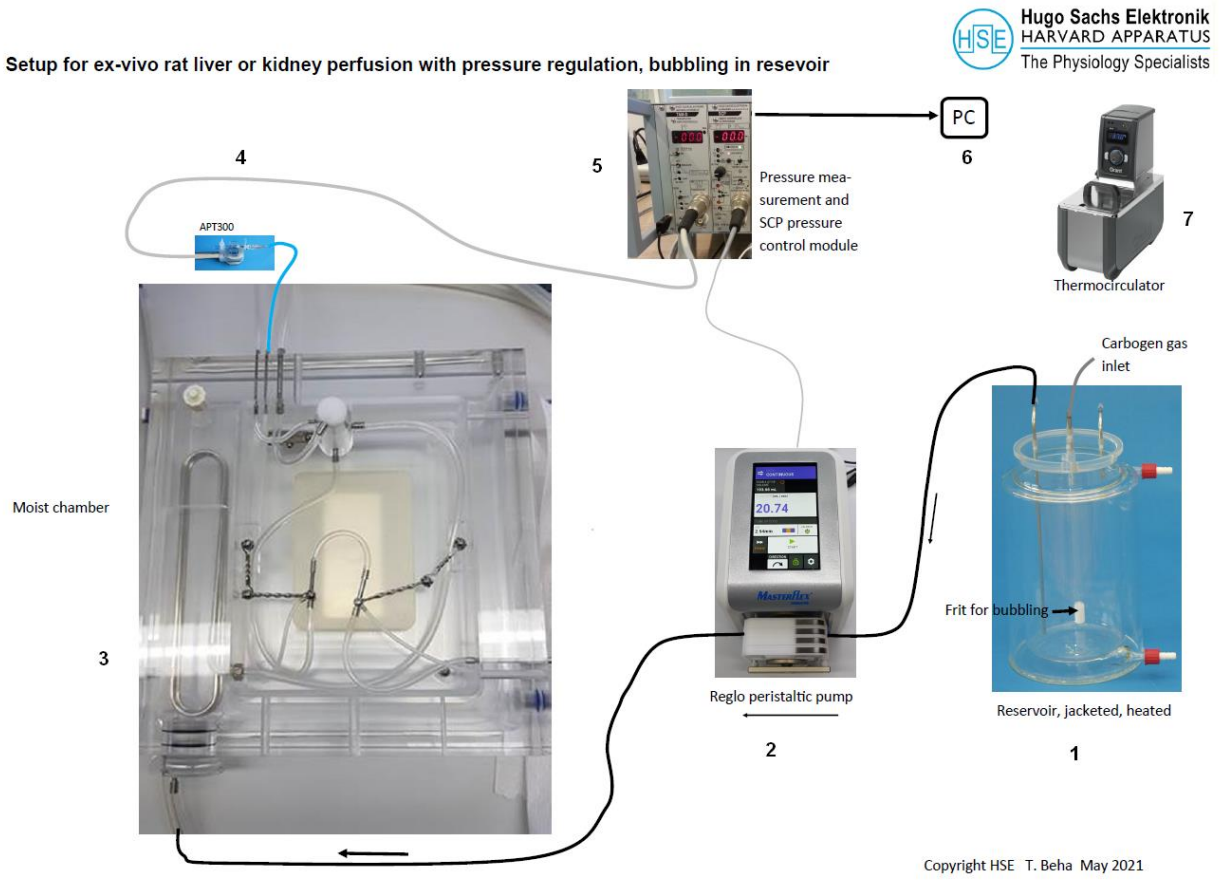


Fig. 10: The Universal Servo Controlled Perfusion System

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1 Buffer reservoir 0.5, 1 or 2 L depending on application and experiment timing
Includes a bubble stone (frit) for bubbling with O2 and CO2, usually carbogen.

2 Peristaltic pump with analog voltage input, controlled by the HSE SCP module (5).

3 Heated moist chamber

4 APT-300 pressure transducer connected in a side branch to measure the perfusion pressure (arterial pressure)

5 PLUGSYS housing with TAM-D and SCP module. The required perfusion pressure or perfusion flow are set on this module. It receives the perfusion pressure signal (actual values) from the HSE TAM-D module.

In constant pressure mode (CONTROLLER MODE) the SCP module continuously compares the actual measured pressure values with the set values (e.g. 80mmHg in kidney perfusion) and controls the pump speed in a way that the pressure remains constant.

In Constant flow mode (DIRECT MODE) the user can set the required flowrate and the SCP module runs the pump at the wished speed. In this mode the TAM-D measures the perfusion pressure which will increase with the pump speed (flow)

6 A PC with Data Acquisition DAQ software can be added to record and evaluate the pressure and flow readings and calculate vascular resistance

7 A thermocirculator is used to heat the chamber and the buffer reservoir

7.3.1 Control system for constant-pressure perfusion in detail

A scheme of a control system for constant-pressure control is shown below, using kidney perfusion as example. The required perfusion pressure (= set pressure = SETPOINT X) is set on the SCP module. The output signal controls the speed, respectively the flow rate of the pump.

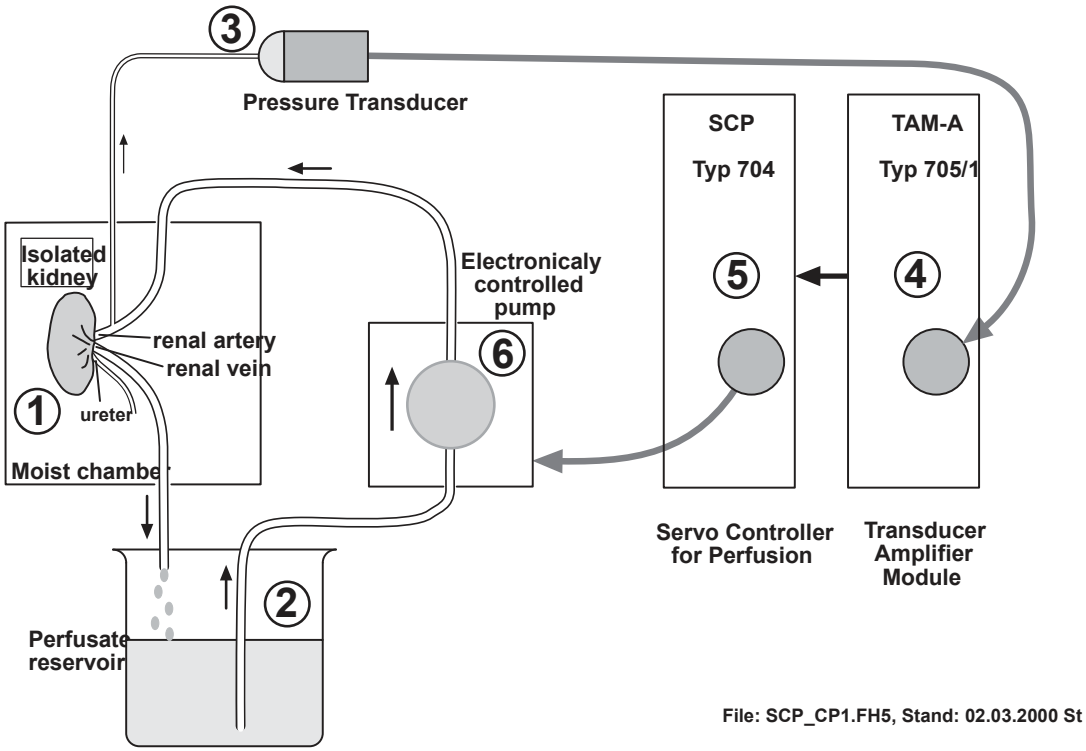
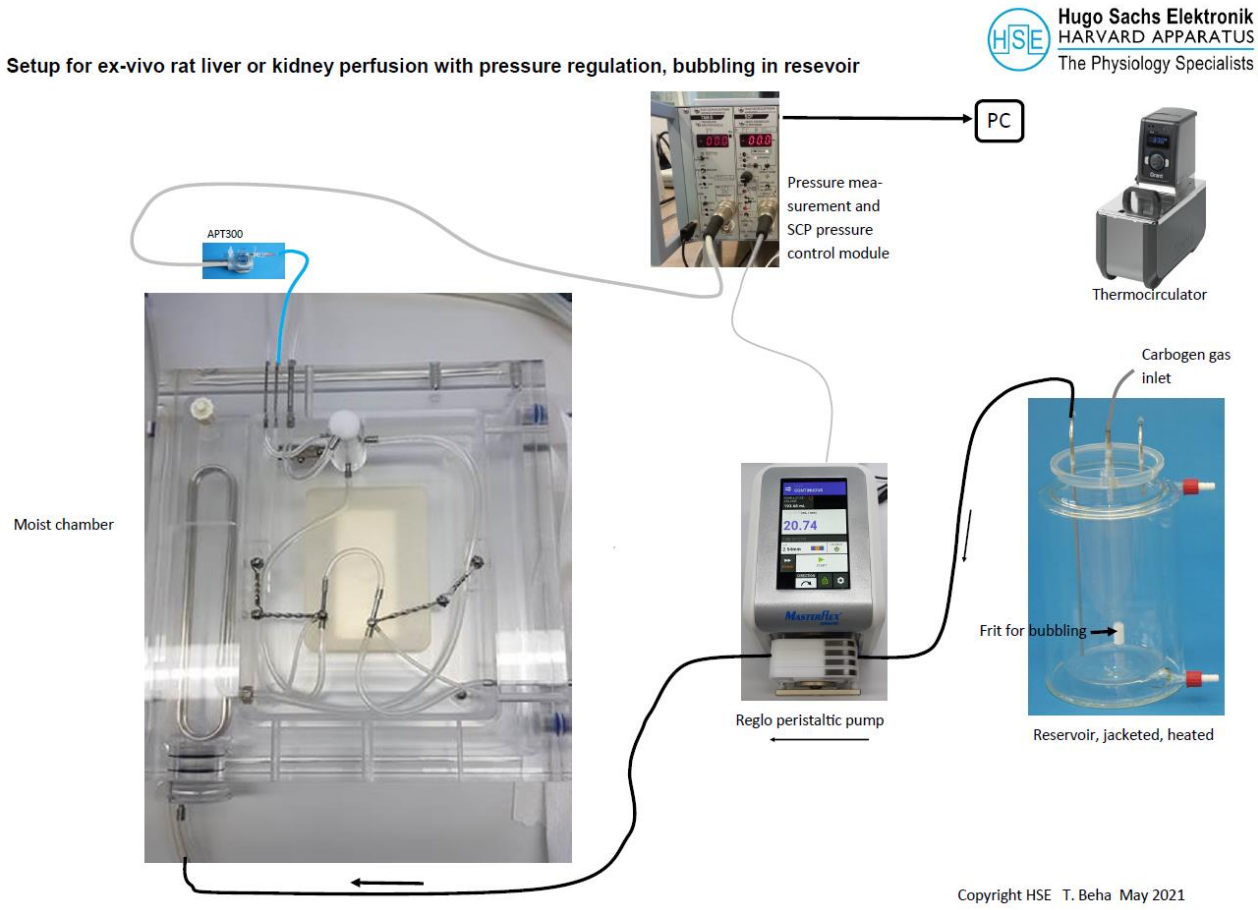


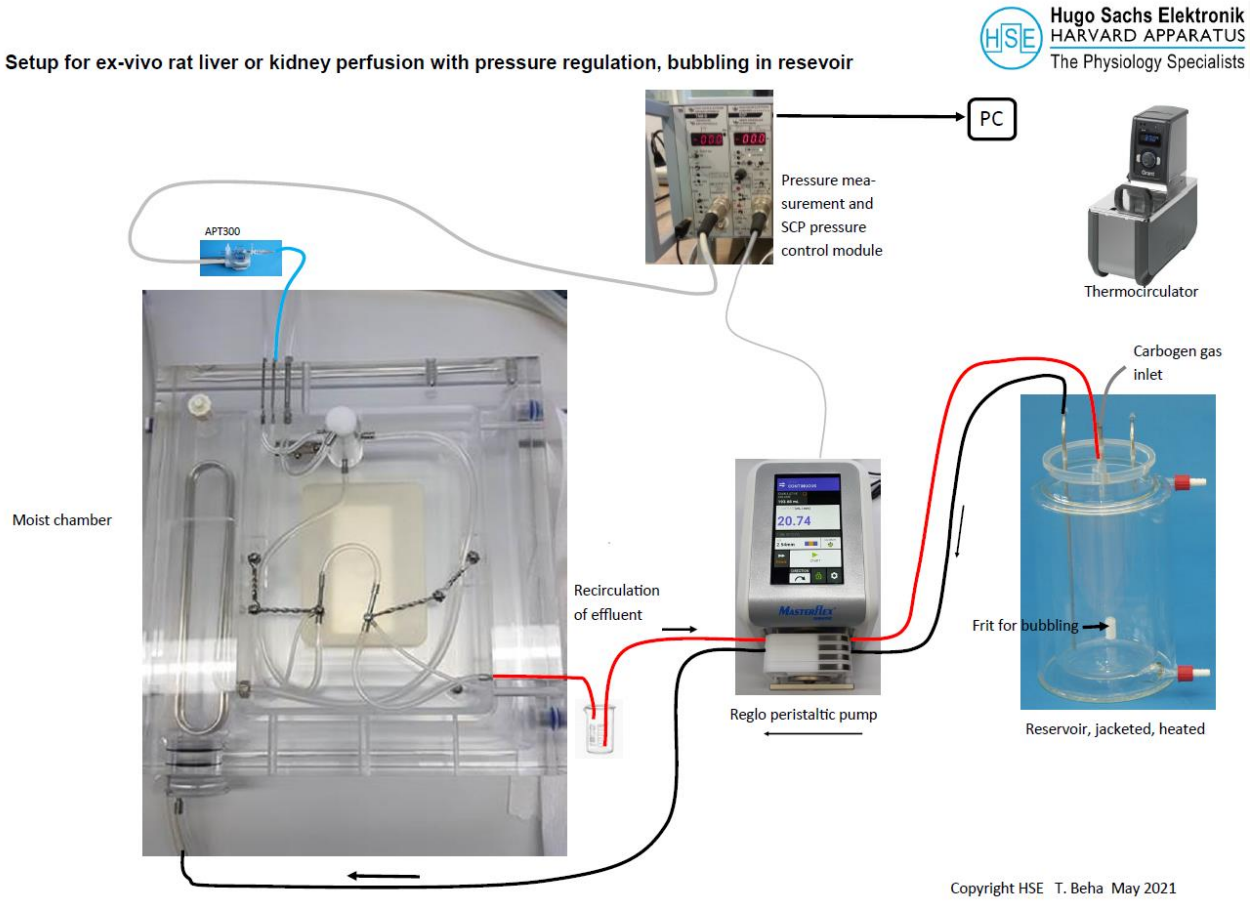
Fig. 11: Control system for constant pressure perfusion.

1 Moist chamber with the isolated organ.
2 Perfusate reservoir.
3 Pressure transducer (e.g. APT-300). The pressure transducer measures the perfusion pressure close to the organ and passes it to the bridge amplifier (e.g. HSE TAM-A or TAM-D, 4).
4 TAM-A module (Transducer Amplifier Module). In combination with the APT-300 pressure transducer the module is used to measure the actual perfusion pressure. The output signal of the TAM corresponds with the perfusion pressure and is passed to the SCP module (5). Instead of the HSE TAM-A module a HSE TAM-D module can be used.
5 The HSE SCP module is a PID controller. It always compares the actual signal received from the HSE TAM-A (4) with the set value and produces a control signal. This signal operates the roller pump (6) in such a way that the perfusion pressure is as closely as possible with the set value. The required perfusion pressure is set on the SCP Module.
6 Peristaltic pump. The peristaltic pump is externally controlled by the SCP module (5) and provides the required flow rate.

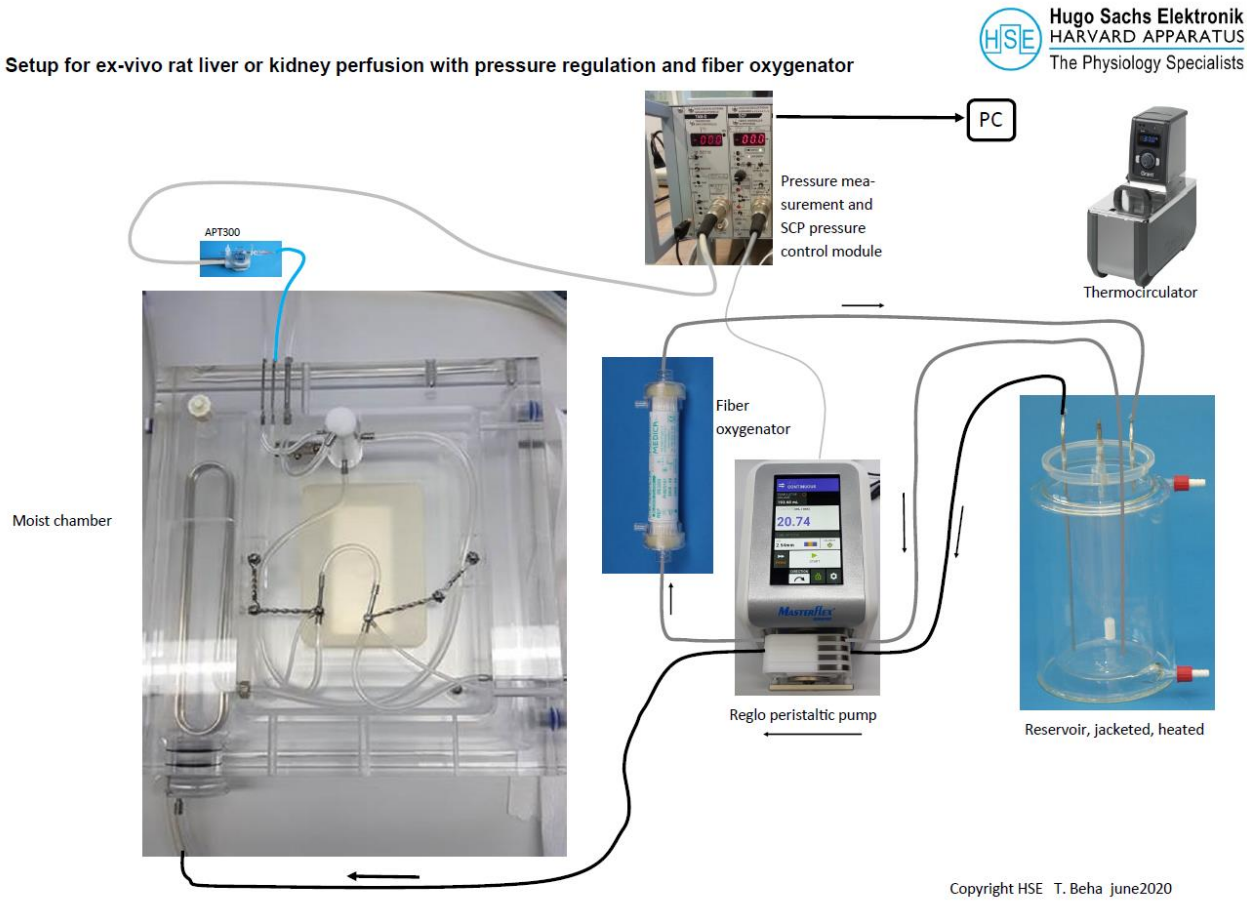
7.4 Setup for ex-vivo rat liver or kidney perfusion with pressure regulation and bubbling in a reservoir



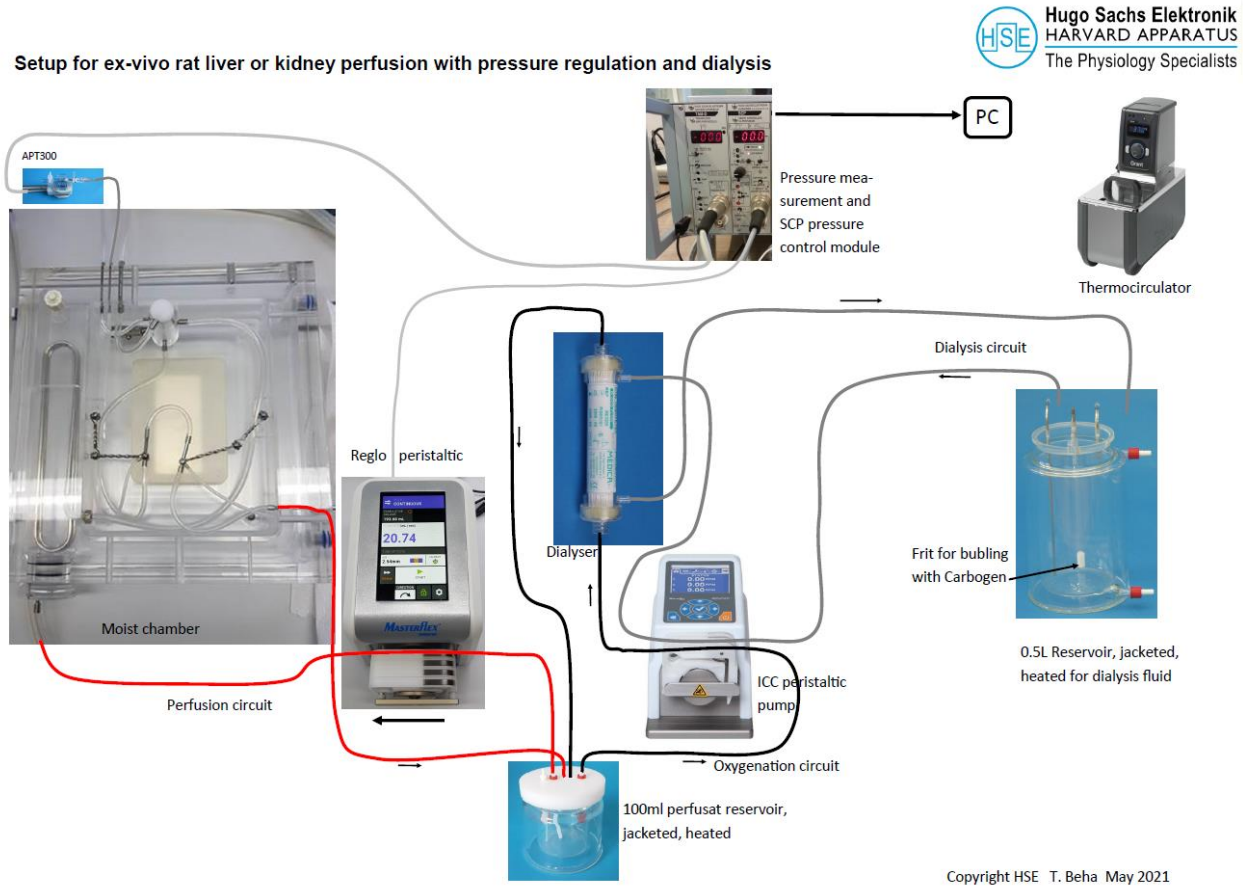
7.5 Setup for ex-vivo rat liver or kidney perfusion with pressure regulation, bubbling in reservoir, recirculating system



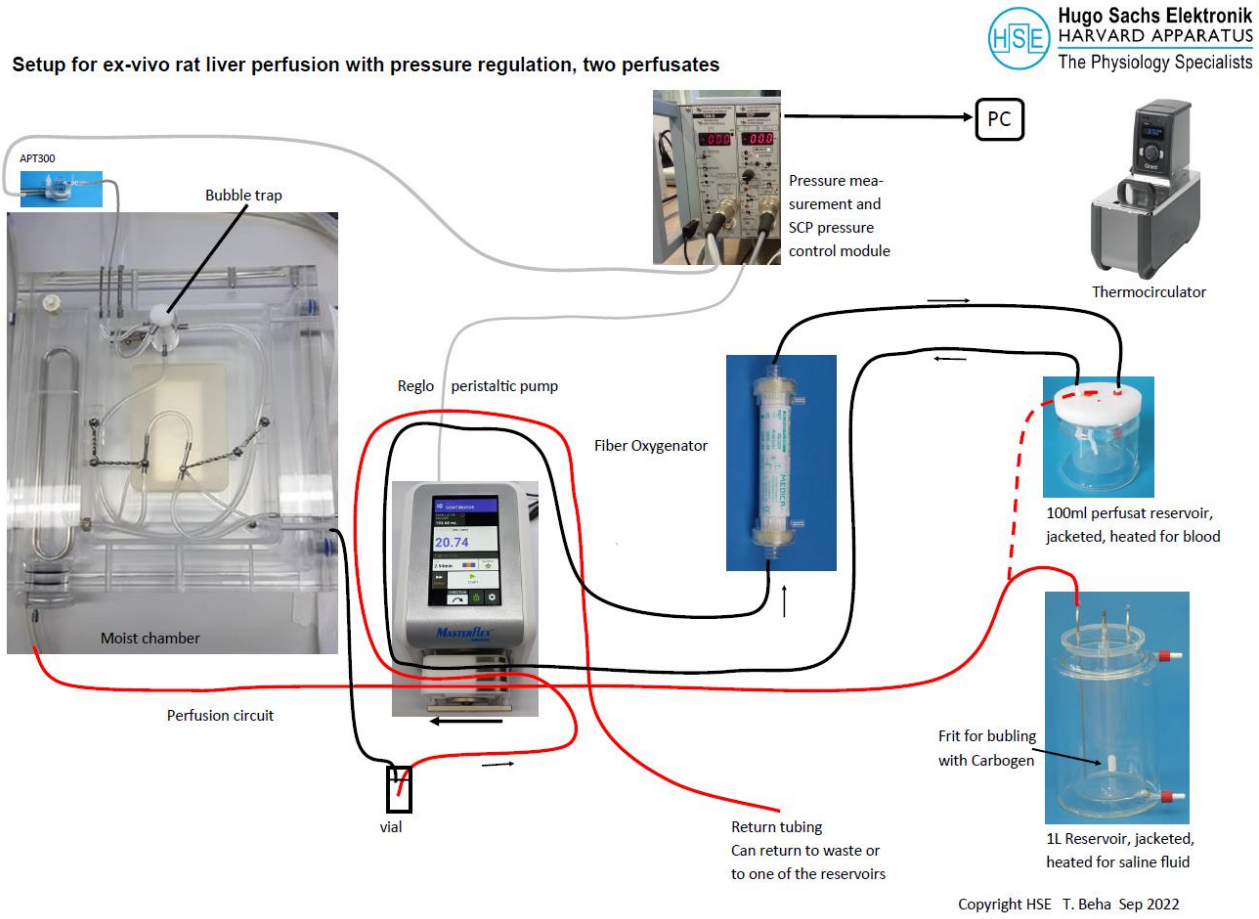
7.6 Setup for ex-vivo rat liver or kidney perfusion with pressure regulation and fiber oxygenator for foaming substances



7.7 Setup for ex-vivo rat liver or kidney perfusion with pressure regulation and dialyser circuit



7.8 Setup for ex-vivo rat liver or kidney perfusion with pressure regulation and fiber oxygenator. Two reservoirs one with bubbling the second with fiber oxygenator



8. Servicing and maintenance of the moist chamber

8.1 Replacing the tubing between heat exchanger and bubble trap

The moist chamber is provided with a metal tube heat exchanger so that adequate warming of the perfusate is ensured even at larger flow rates. The stainless-steel heat exchange is installed to compensate the temperature loss between the heated reservoir and the organ in the chamber. As the heat exchanger is built into the heating circuit inside the chamber, exchanging of tubing is a bit different at this point.

In normal operation when no substances adhering to the tubing walls, are added to the perfusate, this tubing can also be used for a long time before it becomes necessary to replace it (after one year or longer). In case of contamination, it must however be replaced immediately. Whenever you are replacing the tubing carrying the perfusate you should also replace this connection with a fresh piece of tubing.

To replace the heat exchanger tubing you must first drain the thermostatic circuit. Use clamp to block the thermostating tubing. Then pull the heat exchanger out of the bottom part of the chamber. If the heat exchanger stopper has not been removed for a while it may be that the O-ring seals tend to stick. In that case try to loosen the insert by forcefully rotating it combined with pulling. Use a tool only as a last resort (pliers).

IMPORTANT: do not scratch the sealing faces for the O-rings during the manipulation!

Cut off the tubing end (projecting into the chamber) on the inside of the chamber near the squeeze gland in the chamber wall and pull the heat exchanger with the tubing end out of the bore.

Remove the old tubing. Check the O-ring seals for damage. Any O-rings showing cracks should be replaced. If you do not have any new O-rings available, you should immediately obtain replacements and fit them as soon as possible.

[1 replacement O-ring: HSE No. U40069 (dimensions 22 x 1.5)]

A: prepare the new tubing, pointing the end with a scissor, use a long enough tubing see below

B: insert the pointed end of the tubing through the bore into the inner chamber

C: place heat exchanger and tubing into their correct position

First, prepare the new tubing.
You require a piece of Tygon® tubing about 25 cm (10inch) long (HSE No. R43011, ID = 1.52 mm, OD = 3.24 mm). Use only this tubing size! Connect the tubing to the metal heat exchanger tube and form the other end of the tubing to a point with a sharp tool (knife or scissors) (see arrow in **A**).
Then use blunt forceps to introduce the „pointed“ tubing end through the bore of the squeeze gland into the chamber (see arrow in B), grasp the tubing point and pull the tubing through the gland a short distance into the chamber. Do not pull it inside too far! Draw it in only so far that **the tubing piece between the gland and the heat exchanger metal tube is still about 13 - 14 cm** (5.5 inch) long. The tubing outside should not be any shorter so that the heat exchanger can easily be inserted into the bore of the chamber body by a pivoting movement.
Now use a finger to wipe the inner surface of the aperture for the heat exchanger to remove any dirt there; then insert the stainless steel tube of the heat exchanger into the aperture by a pivoting movement. Ensure that you insert the tube underneath the tubing projecting from the squeeze gland. By moving the heat exchanger backwards and forwards and by rotating it axially you should then arrange the tubing so that it comes to rest as a loop above the metal tube without any sharp bends or kinks (see arrow in **C**). If you have left the tubing too long so that it can not be arranged in the desired loop shape you should pull it a little further into the chamber. Do not however pull it in too far so that later at the next tubing replacement you do not have any problem when withdrawing the heat exchanger. When the tubing has been positioned correctly you can push in the Plexiglass stopper up to the stop. Refill the thermostating circuit. Check everything for leakage. If the heat exchanger is not a fully leak tight fit in its aperture you should replace the O-rings. If you do not have any new O-rings available, you can make a temporary seal by applying a little Silicone paste as a thin layer on the contact faces of the O-rings. Do however be certain to obtain new O-rings (see above). Shorten the tube which enters the chamber, so that its length fits to the metallic connector of the bubble trap.

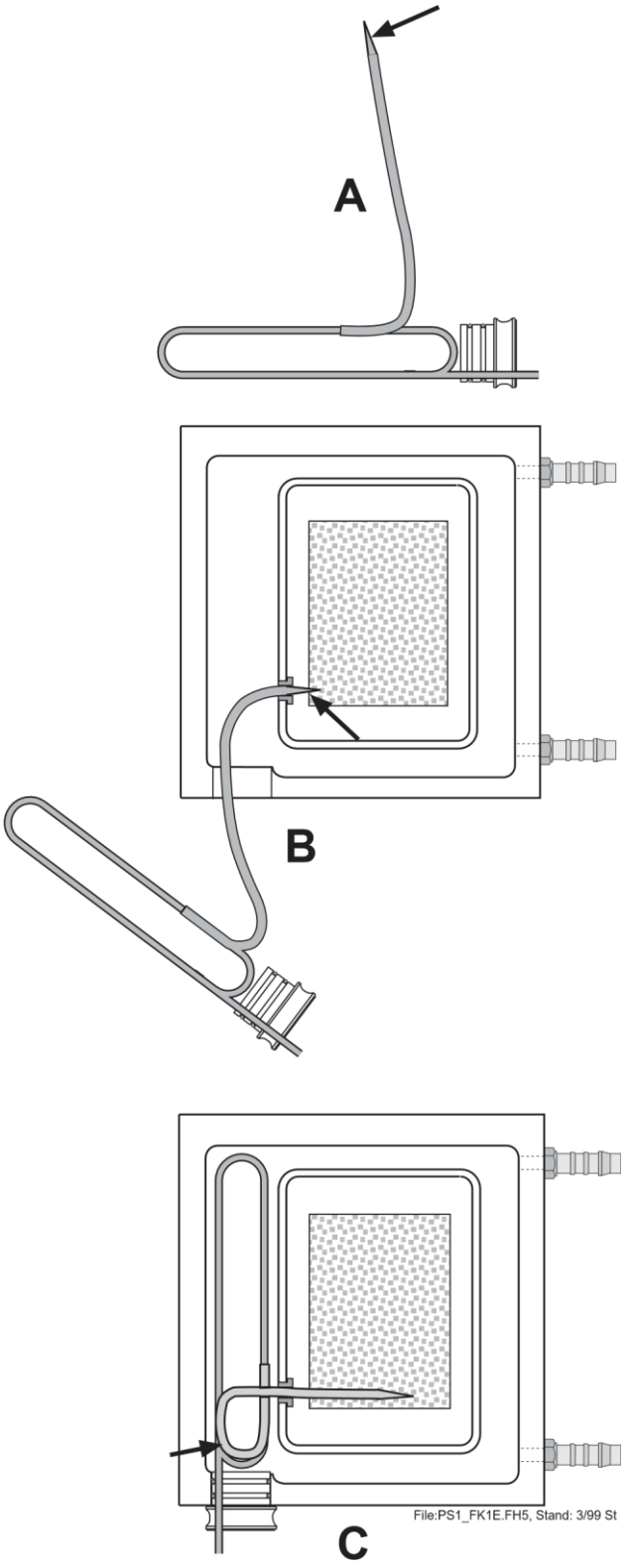
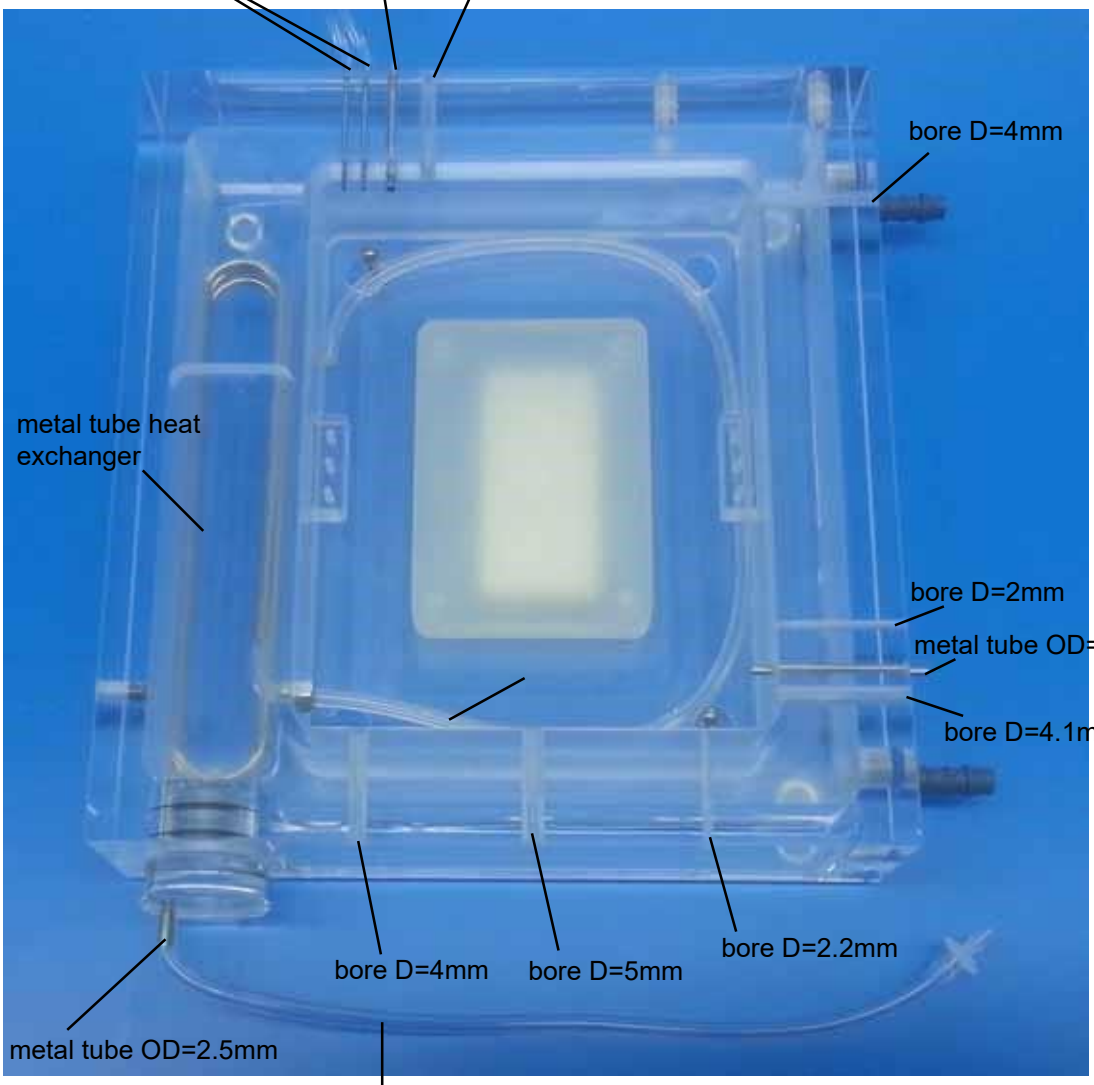


Fig. 12: Replacing tubing from heat exchanger A**: preparation,** B**: insertion,** C**: final position**

8.2 Moist Chamber Type 834/8 bore sizes and tubing diameter

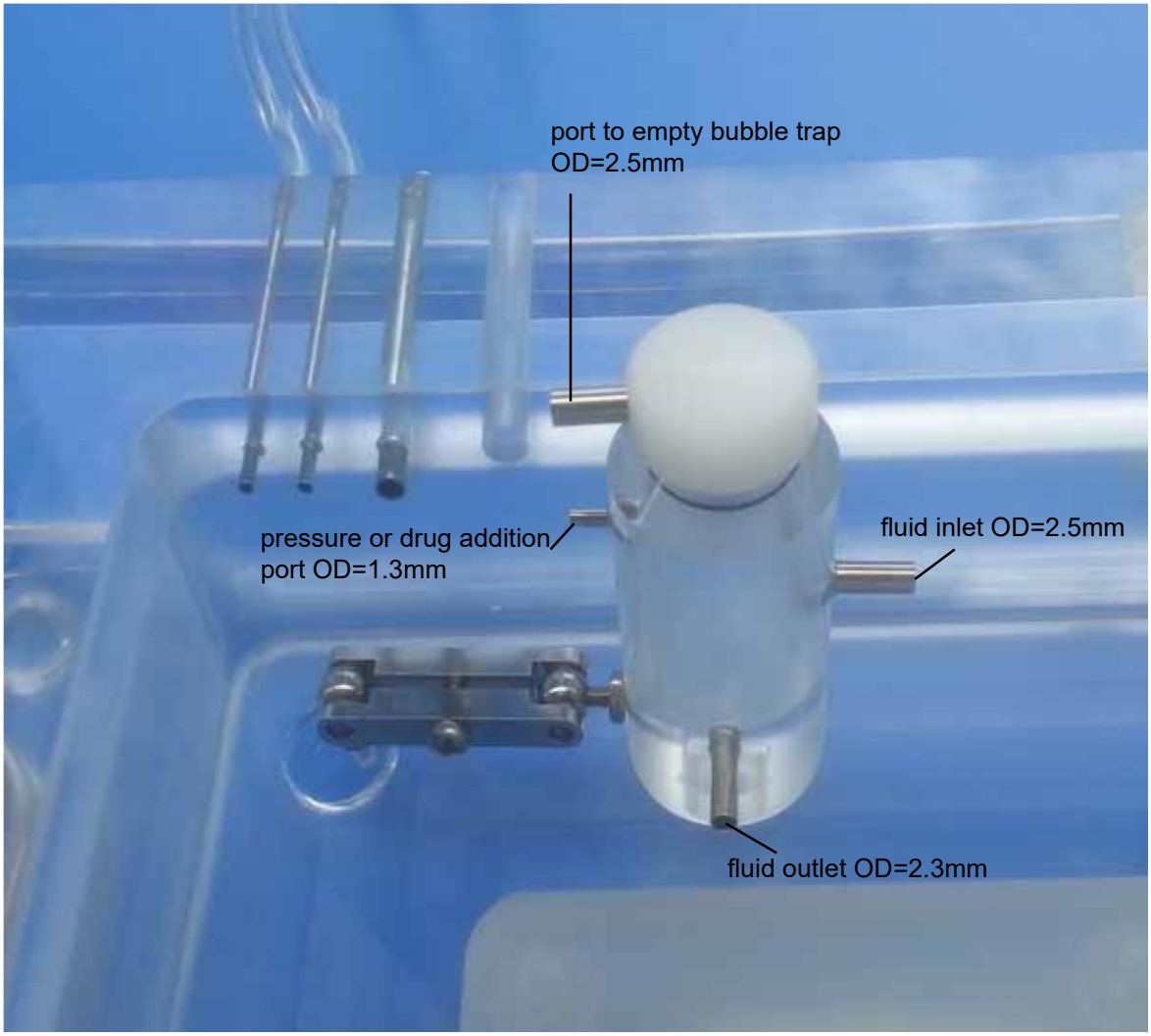


Recommended TYGON tubing:

R43010	OD= 2.86 mm	ID= 1.14 mm	fits on all 1.3 mm stainless steel metal tubes
R43011	OD= 3.24 mm	ID= 1.52 mm	fits on all 2 mm stainless steel metal tubes
R43012	OD= 4.0 mm	ID= 2.0 mm	fits on all 2.5 mm stainless steel metal tubes

Available O-ring and tube kit:
73-5260 SPARE KIT, PERFUSION TUBING and O-RINGS FOR ABDOMINAL ORGAN CHAMBER (MOIST CHAMBER)

8.3 Bubble trap in Moist Chamber Type 834/8, metal tube diameters, tubing diameter



Recommended high flexible Silicone tubing:

R42022	OD= 1.2 mm	ID= 1.8 mm	fits on OD 1.3mm stainless steel metal tubes

Recommended Tygon tubing:

R43010	OD= 2.86 mm	ID= 1.14 mm	fits on all 1.3mm stainless steel metal tubes
R43011	OD= 3.24 mm	ID= 1.52 mm	fits on all 2 and 2.3mm stainless steel metal tubes
R43012	OD= 4.0 mm	ID= 2.0 mm	fits on all 2.5mm stainless steel metal tubes

Available O-ring and tube kit:
73-5260 SPARE KIT, PERFUSION TUBING and O-RINGS FOR ABDOMINAL ORGAN CHAMBER (MOIST CHAMBER)

8.2 General notes on cleaning the moist chamber

Never use alcohol or alcohol containing substance to clean plexiglass

The daily effort involved in cleaning the apparatus depends largely on what substances are being tested. If the substances do not adhere strongly to surfaces (tubing), cleaning can be limited to thorough flushing with distilled water of all parts in contact with the perfusate including the interior of the chamber (the silicone pad can be removed). If this is not sufficient, a cleaning solution such as „**RBS 50**“ or „**Mucaso**l“ (perform classic mucasol) should be employed.

You should make it a regular rule to clean the apparatus thoroughly with the aid of a cleaning solution before each weekend. All parts in contact with the perfusate have to be flushed thoroughly with the cleaning solution. It is recommended that the apparatus is allowed to stand over the weekend filled with cleaning solution. Before starting the experimental work again, the cleaning fluid has to be removed and the apparatus flushed thoroughly several times with distilled water.

Maintenance of the thermostatic circuit is limited to replacing the liquid immediately the growth of algae in the circuit is recognised. By using Thermoclean DC no algae should grow. In addition it is recommended to renew the thermostating liquid regularly every 6 months. Any leakage in the thermostatic circuit should be dealt with immediately. The liquid level can fall in the course of time through evaporation; you should therefore check from time to time whether the distilled water has to be topped up.

It should be obvious that you remove immediately any external contamination through blood or salt-containing perfusion solution. Use a cloth moistened with water for this purpose. For any spot hard to remove you can additionally use a little cleaning agent. **Never use any abrasive cleaner or alcohol !**

Longer shut-down periods:
With longer intervals between the experiments, it is better to leave the moist chamber to stand dry after cleaning. Before the experiment fill it with cleaning solution and allow to act it overnight.
In the morning switch on the thermostat to warm the cleaning solution again. Then remove the cleaning solution from the apparats and flush it thoroughly with distilled water.

8.3 Recommended cleaning agents

Please only use the recommended cleaning solutions! Never use alcohol for cleaning Plexiglass!

Important: not all in a laboratory available cleaning solutions can be used for cleaning Plexiglas ! E.g. MUCOCIT F, supplied from Merz, Frankfurt damages Plexiglas.

To avoid damages **only** use the cleaning solutions recommended from the manufacturer of the apparatus.

RBS 50 bzw. RBS 35
Manufacturer and supplier:
Carl Roth GmbH + Co KG, Chemische Fabrik,
Schoemperlenstr. 1-5, D-76185 Karlsruhe
Phone: +49 (0) 721/5606-0, Fax: +49 (0) 721/5606-49,
htttp://www.carl-roth.de

MUCASOL (trade name change to “perform classic mucasol”)
Manufacturer: Merz + Co. GmbH & Co. Bereich Dr.Kramer,
Eckenheimer Landstrasse 100-104, D-60318 Frankfurt/Main
Phone: +49 (0) 69/15031, Fax: +49 (0) 69/5962150

Supplier: Firma Rudolf BRAND GmbH & Co.
P.O.Box 11 55, D-97861 Wertheim,
Phone: +49 (0) 9342/808-0, Fax: +49 (0) 9342/808-236

Supplier USA: HARVARD Apparatus, Holliston, MA, ordering number 73-2642

CIDEX® OPA
Supplier in Germany
Johnson & Johnson MEDICAL GmbH
Robert-Koch-Str. 1
22851 Norderstedt
Telefon-Nr. (+49) - (40) - 5297 - 01
Fax-Nr. (+49) - (40) - 5297 - 5379

To avoid algal growth in the water bath we recommend:

THERMOCLEAD DC
Manufacturer: BIORAPID GMBH, Waldmatten 13, 79224 Umkirch, Germany

Supplier HSE or HA USA, ordering number 73-4246 (10ml) or 73-4261 (100m dosing bottle)

More information you can get from the document **General cleaning recommendations for HSE Perspex perfusion systems IH-SR, IH-5, IPL-1, IPL-2, IPL-4, moist chambers etc.** which you have got with your system

9. Tubing material

The tubing used in the apparatus has to meet various requirements. Appropriate tubing materials have to be selected so that their properties best meet the requirements. In addition to chemical, mechanical and optical properties it is also necessary to take account of the gas permeability of the various materials. The selection of certain materials as listed below is intended to be only a general guide. The user himself must decide which type of tubing is most suitable for his particular application. In case of doubt, it is necessary to carry out tests and make appropriate measurements.

When considering permeability to gas, the time the liquid remains in the tubing is of course quite important. Longer periods result in more gas exchange with the surrounding air than in the case of short periods. In case of doubt, it is necessary to carry out appropriate measurements.

Silicone tubing:
Silicone is the tubing material most widely used in the laboratory. It is largely inert and resistant to most chemicals used in the laboratory. Natural silicone is not glass clear but is sufficiently translucent so that the lumen can be examined visually. A further advantage are its favourable mechanical properties. When used in roller pumps there is relatively little wear.

Silicone exhibits a high permeability to oxygen. Therefore: do not use Silicone tubing when you must avoid oxygen interchange between the atmosphere and the tubing lumen!
Silicone tubing is not recommended for perfusion tubing, use Tygon which is 60 times less permeable to O2 and CO2

Tygon® (R-3603):
Tygon® is mostly used in the laboratory. It is manufactured under strictly controlled conditions. It is largely inert and resistant to most chemicals used in the laboratory. Tygon is glass clear and permits optimum visual

checking of the lumen. Tygon tubing is flexible, and the remaining mechanical properties are also favourable. When used in roller pumps it suffers somewhat more wear than Silicone.

Tygon has a low permeability to oxygen. It is therefore a better tubing material than Silicone when any exchange of oxygen between the surroundings and the lumen has to be avoided. Thick-walled tubing is preferable to thin-walled tubing in this case.

PharMed™ :
PharMed™ tubing has been specially developed for medical applications. Chemical and mechanical properties are excellent. Its permeability to oxygen is practically zero. The disadvantage is that the material is not transparent. Visual examination of the lumen is not possible.

10. Chemical behavior of PLEXIGLAS®

The data given refer to a test temperature of 23° C and presuppose stressfree installation. The behavior of the material in practice depends largely on the temperature in use. In case of doubt, we advise you to consult us as to the chemical resistance for particular applications.

The results obtained for all products, especially the branded ones, refer to production batch tested in each case.

The symbols signity:

+ resistant
- not resistant
o limited resistance

Antistatics :

+ HB 155
+ Antistatic fluid and cleaning agent

Technical baths :

+ Electroplating baths
+ Photochemical baths

Chemicals, solvents, etc.

a) General

- Acetic acid, concentrated o Acetic acid, up to 25 %
- Acetone
+ Alum
+ Aluminium chloride
+ Aluminium oxalate
+ Aluminium sulphate
- Ammonia water
+ Ammonium sulphate
- Amyl acetate
- Aniline
+ Arsenic
+ Arsenic acid
+ Battery acid
- Benzaldehyde
+ Benzine, pure
- Bromine
- 1-Butanol
- Butyl lactate
+ Butyric acid, up to 5 %
+ Calcium chloride
+ Calcium hypochlorite - Carbon disulfide
- Carbon tetrachloride
- Chlorinated hydrocarbons - Chlorine, liquid o Chlorine water - Chloroethyl ether - Chlorophenol o Chromic acid
+ Citric acid, up to 20 %
+ Copper sulphate
- Cresol
+ Cyclohexane
- Diacetone alcohol o Diamyl phthalate - Dibutyl phthalate
+ Diethylene glycol
- Dioxane - Ether
- Ethyl acetate - Ethanol, concentrated o Ethanol, up to 30 % - Ethyl bromide - Ethyl butyrate
- Ethylene bromide
+ Ferric chloride
+ Ferrous chloride
+ Ferrous sulphate + Formic acid, up to 2 % o Formic acid, up to 40 %
+ Glycerol
+ Glycol
+ Heptane
+ Hexane
+ Hydrochloric acid
+ Hydrofluoric acid, up to 20 %
+ Hydrogen peroxide, up to 30 %
+ Iodine, metallic
+ Lactic acid, up to 20 %
+ Magnesium chloride
+ Magnesium sulphate
+ Manganese sulphate
+ Mercury
- Methanol, concentrated o Methanol, up to 30 % - Methyl ethyl ketone
- Methylated spirits + Milk of lime
+ Monobromonaphthalene
+ Nickel sulphate
+ Nitric acid, up to 40 %
+ Nitric acid, over 40 %
+ Oxalic acid
- Perchloroethylene + Petroleum
+ Petroleum ether
- Phenols
+ Phosphoric acid, up to 50 % - Phosphorus trichloride - Phosphorus, white
+ Picric acid, 1 % in water + Potassium bichromate
+ Potassium carbonate + Potassium chloride + Potassium cyanide
+ Potassium hydroxide solution
+ Potassium nitrate + Potassium permanganate o 2-Propanol + Propylene
- Pyridine
- Silicon tetrachloride
+ Silver nitrate
+ Sodium bisulfite
+ Sodium carbonate
+ Sodium chlorate
+ Sodium chloride
+ Sodium hydroxide solution, 30 %
+ Sodium hypochlorite
+ Sodium sulphate
+ Sodium sulphide
+ Stannous chloride
+ Stearic acid
+ Sulphur
- Sulphur dioxide, liquid + Sulfuric acid, up to 30 % o Sulphurous acid, conc. + Sulphurous acid, up to 5 % + Sulfuryl chloride
+ Tartaric acid, up to 50 %
- Thionyl chloride
- Toluene
+ Triethylamine
- Trichloroacetic acid
+ Turpentine
+ Turpentine substitute
+ Urea, up to 20 %
- Xylene
+ Zinc sulphate, aqueous
+ Zinc sulphate, solid

b) Branded products:

+ ® CLOPHEN T 55,A60
o ® DEKALIN
o ® FRIGEN A 12(CF_2 CL_2)
- ® GLYBAL A + ® PALATINOL K o ® PALATINOL O, BB new
+ ® SANGAJOL
+ ® TERAPIN
- ® TETRALIN

Disinfectants

a) General

- Carbolic acid
+ Chlor. lime paste

- Hydrogen peroxide, up to 40 %
o Hydrogen peroxide, over 40 % - Iodine tincture, 5 %
+ Lugol solution
- Methylated spirits
+ Sublimate

b) Branded products

o ® ÄTHROL, up to 5 % + ® BAKTOLAN, up to 5 % - ® BAKTOLAN, conc.
+ ® CHINOSOL, up to 1 %
- ® CHLORAMIN, suspension
+ ® CHLORAMIN; solution
+ ® ELMOCID GAMMA, up to 2 %
- ® LYSOFORM
+ ® MEFAROL, up to 1 %
+ ® MERCKOJOD, up to 1 %
+ ® MERFEN
+ ® PERHYDROL
+ ® PERODIN
+ ® SAGROTAN, up to 2 % o ® SAGROTAN, up to 5 % o ® VALVANOL, up to 2 % + ® ZEPHIROL; up to 5 %

Fats, oils, waxes :

+
Animal +
Mineral
o Silicone oil +
Vegetable

Gases and vapours

+ Ammonia
o Bromine vapours, dry + Carbon dioxide
+ Carbon monoxide
+ City gas
o Chlorine vapours, dry + Exhaust gases containing HCl + Exhaust gases containing HF
+ Exhaust gases containing H_2SO_4
+ Hydrogen sulphide
+ Methane
+ Nitrogen dioxide
+ Nitrogen monoxide
+ Oxygen
+ Ozone
+ Sulphur dioxide, dry

Beverages, etc.

+ Beer, Wine
+ Camomile extract + Chocolate
+ Fruit juice, milk, coffee
o Spirits, up to 30 %
+ Vinegar
+ Water, mineral water

Cosmetics, etc.

- Camphor
+ ® DIPLONA -hair oil
+ Face tonic
+ Glycerine
+ Hair setting lotion (PRIMAWELL)
- Nail varnishes
- Nail varnish removers
+ Ointments
+ Peat water
+ ® POLYCOLOR
+ Seawater +
Soaps o Sprays

Plastics

+ Foam plastics
- Foam plastics, plasticised
+ Polyamide
+ Polyethylene
+ PVC
- PVC, plasticised
+ Rubber
- Rubber, plasticised

Foods and spices

+ Aniseed, bay leaf, nutmeg
- Cloves
+ Common salt
+ Honey, pure
+ Ice cream +
Meat, fish
+ Pepper, cinnamon, onions
+ Pickles

Cleaning agent

a) General

Acids, see under chemicals - Alcohol, concentrated o Alcohol, up to 30 %
Alkalis, see under chemicals
+ Ammonia solution
- Benzine, mixture, containing aromatics
+ Benzine, non-aromatic
+ Bleach
- Carbon tetrachloride - Methylated spirits
- Perchloroethylene + Petroleum
+ Petroleum ether +
Soap solution
+ Soda water
- Stain remover
- Trichloroethylene + Turpentine + Turpentine substitute

b) Branded products

+ ® AJAX
+ ®Antistastischer KUNSTSTOFF-REINIGER und Pfleger
+ ® BFK cleanser
+ ® BOLIMENT
+ ® BÖTTCHERIN
+ ® BURMAT
+ ® BURNUS
+ ® CILLIT-GRÜN
+ ® DOR
+ ® DOSYL
+ ® DOSYLAN
+ ® FAKO-Polish
+ ® FAKO-Polishing paste
+ ® FEWA
+ ® FRAPPIN
+ ® FÜLLBOX
+ ® LAWAPLEX
+ ® NULL-NULL
+ ® PERSIL
+ ® PLEXIKLAR
+ ® PRIL
+ ® REI
+ ® SEIFIX
- ® SIDOLIN
- ® SPECTROL
+ ® SPÜLI
+ ® WC-00

c) Cleaning agents for pipes and tanks

+ ® CALGONIT D, DA, S
+ ® NEOMOSCAN M, M powder
+ ® NIROKLAR GR liquid
+ ® NIROKLAR GR powder
+ ® P 3
o ® P 3 basic cleaner
+ ® P 3- dix

Pesticides

- Sprays (applied directly)
o Sprays (applied in the air) o Pesticides in aqueous solutions
+ ® NEXION stable spray
+ ® RABOND stable spray

Protective coatings (strippable)

+ ® DIEGEL liquid film 23922
+ ® KOPPERSCHMIDT covering paste o ® SPRAYLAT

Other substances

+ Urine
- Fuel for petrol engines o Fuel for diesel engines