



AtmosLM Large Molecule Sampling Manual

1. System Set Up

Figure 1 shows a typical AtmosLM setup. A syringe pump is used to infuse perfusate and the peristaltic pump is used to withdraw sample from the probe. Always keep the peristaltic pump (ERP-10) at the same level as, or not more than 50cm above, the AtmosLM probe to avoid reduced flow from the outlet line of the probe.

Name	Model	Specification
Syringe Pump (Push Pump)	ESP-32	1 motor 2 drive
	ESP-64	2 motor 4 drive
Peristaltic Pump	ERP-10	2 channel
Fraction Collector	EFC-82	Main
	EFR-82	Cooling Unit
Freely Moving System	ATFM-RA	for rats
	ATFM-MA	for mice
AtmosLM Probe	PEP-X-Y	Polyethylen 1,000 kDa

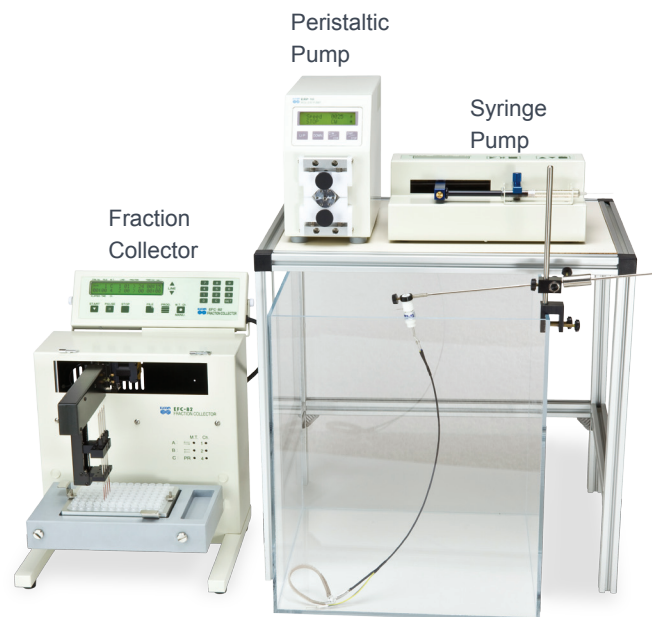


Fig. 1 An Example of System Setup

2. Operation of Peristaltic Pump ERP-10

One RS-5S Peristaltic Tubing can be installed on each side of the roller, upper and lower. Tubing Guide is the groove where the peristaltic tubing, RT-5S, is placed. To move the tubing into place against the rollers, please pull and slide the Knob until you hear a click. The rotation speed shown in the display is NOT the RPM or flow rate, but represents an indication of the rotation speed of the roller head. When you use the RT-5S tubing and set the speed to "0020", the actual flow rate will be 1 μ L/min. Do NOT place the ERP-10 higher than approx. 50 cm, =20 inches above the probe to avoid the flow fluctuations. In order to prolong the life of the RT-5S, please open the Tubing Guides such that they don't compress the tubing whenever the pump is being stored.

Buttons and Actions

Up	Increases the rotation speed of roller.
Down	Decreases the rotation speed of roller.
CW/CCW	Selects the direction of roller rotation, CW indicates Clock Wise and CCW is Counter Clock Wise. If held down for 3 seconds, the rotation speed will be set to 500. If held for 5 seconds, it will be to 1000.
Start/Stop	Starts or Stops the rotation of roller. If held down for 5 seconds, the input value will be memorized and the same value will return after you reboot the unit.

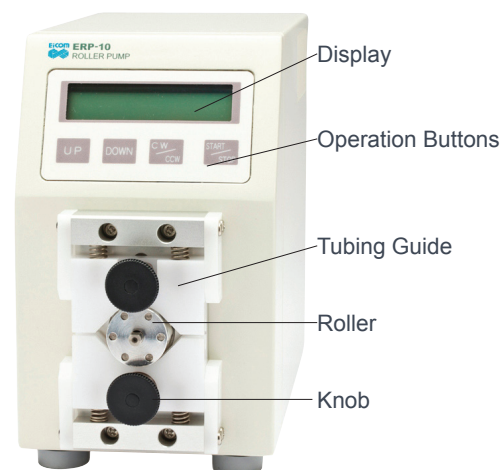


Fig. 2 Peristaltic Pump ERP-10

3. Tubing Setup

Connect all tubing as shown in Figure 3. Table 1 shows the model names of tubing, size, material and approximately internal volume of each tube shown in Fig.3. The tubing between the probe and peristaltic pull pump has to be 0.25 mm ID to allow smooth withdrawal of the sample.

4. Peristaltic Tubing

Please always use Eicom RT-5S for the tubing set on the ERP-10. Please exchange RT-5S to new one at least every two (2) weeks if you use every day. The lifetime for storage is 6 months. When you do not use the ERP-10, please open the tubing guide to relieve the pressure on the RT-5S.

RT-5S Includes

Peristaltic Tubing	5 pcs
Joint (#RTJ)	4 pcs
Internal Diameter	0.25 mm
Total Length	83 mm

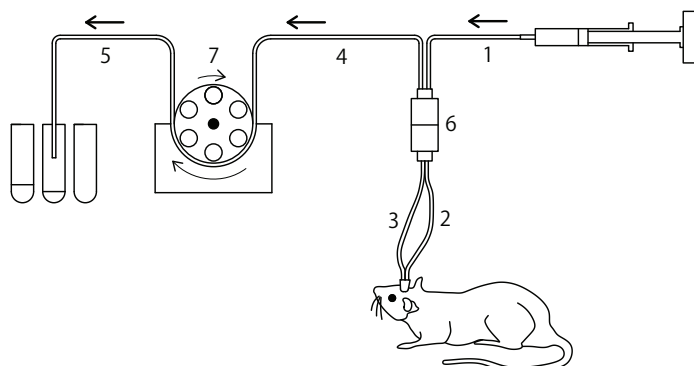


Fig. 3 Tubing Diagram

Details of each tubing and parts on figure is explained on Table 1. Please find corresponding number in the table and this figure.

Table 1. Information of Each Tubing

	Model Name	Size, Material	Approx. Volume
1. Tubing from Syringe to Swivel	JT-10-50	0.1 mm ID x 50 cm, Teflon Tubing	4 µL
2. Tubing from Swivel to Probe	Rat WT-35-40	0.1 mm ID x 40 cm, FEP	3.1 µL
	Mouse WT-25-25	0.1 mm ID x 25 cm, FEP	0.6 µL
3. Tubing from Probe to Swivel	Rat WT-35-40	0.25 mm ID x 40 cm, FEP	20 µL
	Mouse WT-25-25	0.25 mm ID x 25 cm, FEP	12 µL
4. Tubing from Swivel to ERP-10	JF-10-50	0.25 mm ID x 50 cm	25 µL
5. Tubing from ERP-10 to Fraction Collector Needle	JT-10-50	0.1 mm ID x 50 cm Teflon	4 µL
	+ FRN-JJ	+ 0.12 mm ID x 11 cm PEEK	+ 1.3 µL
6. Liquid Swivel	TCS2-21	0.45 mm ID for both in and out, Stainless Steel 316	In 11 µL Out 10 µL
7. Peristaltic Pump Tubing and Joints	RT-5S RTJ	Tygon (Vinyl chloride) 0.25 mm ID x 83 mm Stainless Steel 26G x 15 mm	Total 6 µL
Joint Tubing	JB-30	Biton 0.3 mm ID x your cut	Vary

Customized tube lengths are available for JT-10, JF-10 and WT-35.

5. You will need:

aCSF, BSA (Bovine Serum Albumin), 0.2 µm syringe filter, one 5 mL plastic syringe, one 2.5 mL or 1 mL disposable syringe, ethanol

6. Flushing System with Water

Flush tubing with water to confirm the flow. At this point, please bypass AtmosLM probe by using a small connector to join the tubing where the probe will be located. If the traveling time of sample is important, we recommend checking the actual traveling time on your system setup. Insert an air gap in the tubing and run the system. Many syringe pumps have a lag time between when the pump is started at the controller and when the actual flow begins. This is because there is a play in the cam and motor gear. So, please first fast forward pump and then switch to normal flow rate before connecting the tubing.

7. aCSF 0.15% BSA

Prepare aCSF containing 0.15% BSA (Bovine Serum Albumin) on the day of sample collection. Please do not include buffer components such as carbonate or phosphate in aCSF. It will have more chance to clog in the line during *in Vivo* use. Filter aCSF with a 0.2 µm membrane filter and fill a Gastight Syringe using on a syringe pump.

8. Flushing System with aCSF

A connector is still being used in place of the probe. Run both the syringe pump and peristaltic pump at 10 times the normal sampling flow rate to fill the system. To set the peristaltic pump at 10 µL/min, please enter "0200" to the speed. After filling all lines without the probe with aCSF/0.15% BSA, stop both pumps at the same time.

9. Quality Check of Probe

Connect a disposable plastic syringe filled with pure/distilled water to the **OUTLET (=shorter needle)** of the probe. Only use a 1mL or 2.5 mL syringe. Larger syringes can easily generate too much pressure. Then cover the probe's vent holes with your fingers and depress the syringe plunger gently to fill the probe. Water should be coming out from Inlet needle. Confirm that there are no leaks from tip of membrane or connecting parts of membrane or shaft. The surface of membrane may have sweat, which is normal.

10. Activate Membrane

Submerge the membrane in ethanol (70% to 100%, it does not matter) for **1 second**. Do NOT keep the probes in ethanol for longer than 5 seconds as this can damage the probe by weakening the glue. The membrane is properly wetted when the opaque white membrane becomes slightly translucent. Now use the plastic syringe to push water into the probe again.

11. Connecting Probe to System

Both the syringe and peristaltic pumps should still be stopped and flow rate settings of the syringe pump is at 10 µL/min and ERP-10 is showing "0200". Remove the bypass where the probe will be placed and connect the inlet and outlet. Start the syringe pump and start the peristaltic pump immediately after confirming a little leak from the vent holes. Pay attention there might be a lag time on the syringe pump caused by a mechanical play, see section 6. Confirm that an air segment no larger than approx. 5 cm (= 2 inch, 2.5 µL) has entered the outlet tubing. (see #3, #4 or #5 on Fig. 3).

12. Insertion of Probe

Maintain the 10 µL/min flow rate and insert the probe into the animal via guide cannula that has been implanted in advance. If dummy probe is hard to remove, please twist and pull. Maintain the higher flow rate for 2-3 hours in order to prevent clogging of the tubing. Then lower the flow rate to 1 µL/min or your desired flow rate. If necessary, refill the syringe of the syringe pump or switch the syringe by using a liquid switching valve such as the SI-60.

Tips

- Set the flow on the peristaltic pump 5% higher than syringe pump. "0021" (=1.05 µL/min) for the peristaltic pump when 1 µL/min on the syringe speed.
- When you lower the flow speed, stopping or lowering the peristaltic pump first and then do the same on the syringe pump to avoid a large air segment in the outlet tube which leads a difficulty of withdrawing sample.
- Clogging mostly happens on the tubing #3 or outlet of the probe after the vent. if it happens. To fix, please disconnect the swivel and replace to a 1 mL syringe containing water at the tubing #3 out (see Fig.3 and Fig 4). Then infuse water toward the probe via tubing #3. The flush will come out from the vent and you can use water.

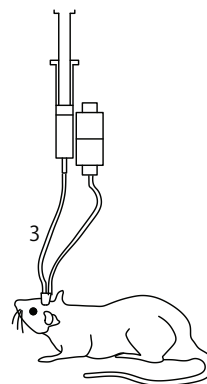


Fig. 4 Removing Clog

13. Maintenance

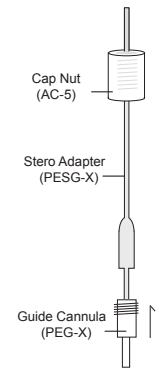
After completing sampling, remove the probe from the system and bypass where the probe was. Open the Tubing Guide on the ERP-10 to prevent tubing occlusion and excess wear of the tubing. Run 50-100 times diluted chlorine bleach in the entire tubing from the syringe pump to the fraction collector and let it stand for at least 15 min. Then disconnect all tubing and flush each piece of tubing with water. If you plan on storing the tubing for more than a few days, run ethanol and then air through the tubing, and store them dry.

Appendix 1. AtmosLM Guide Cannual Implant

Stereotaxic Appratus Adapotr (PESG-X), Guide Cannual (PEG-X), Dummy Cannual (PED-X), Anchor Screw (AN-3), Cap Nut (AC-5)

1. Set Guide Cannual on Adaptor and Fix with a Cap Nut.

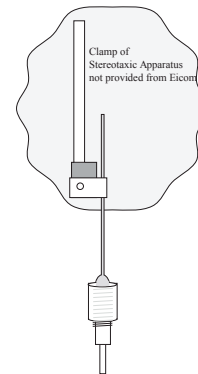
Nut.



2. Clamp the PESG-X

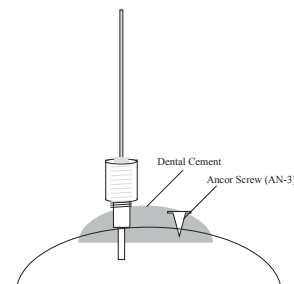
Please use standard electrode clamp which is provided from your stereotaxic apparatus manufacturer. Eicom does not carry this part.

Model Number. Kopf 1770, 1771, 1773. Stoelting 51631, 51632, 51634.



3. Mount Cement

Guide cannula crown is made of acrylic resin and fuse with cement. Cap Nut does not. Please mount dental cement below the thread of the guide cannula.



4. Replace Adaptor with Dummy Cannula

After confirming the cement is dry and firm, loose the cap nut and remove the PESG-X from the PEG-X guide cannula. Then put PED-X dummy cannula in to the guide and fasten the cap nut.

