



AtmosLM Pressure Canceling Microdialysis

1. Tubing Connection and Size

Use **0.25 mm internal diameter** FEP tubing between the AtmosLM probe outlet and the swivel (WT-50), and between the swivel outlet and the peristaltic pump (JF-10). Other connections can be made with 0.1 mm ID (JT-10) or 0.25 mm ID tubing. WT-35 which has 0.1 mm ID for inlet and 0.25 mm ID for outlet is also available as alternative of WT-50.

2. Flushing System

Flush tubing with water to confirm the flow. At this point, please bypass AtmosLM probe with using a small connector to join the tubing where the probe will be located.

3. aCSF 0.15% BSA

Prepare aCSF containing 0.15% BSA on the day of sample collection. Filter it with a 0.2 μm membrane filter before use. Now fill a Gastight syringe with aCSF/0.15% BSA (from step 3) and connect it to the inlet tubing. 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 $\mu\text{L}/\text{min}$, please enter "0200" to the speed. The probe should not yet be connected.

4. 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 probes vent holes with your fingers and depress the syringe plunger gently to fill the probe. Confirm that there are no leaks around membrane.

5. Activate Membrane

Then submerge the membrane in ethanol (70% to 100%, it does not matter) for **2 seconds**. Do NOT keep the probes in the ethanol for longer than 2 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 syringe to push water into the probe again. Then quickly connect the probe to the system and start the syringe pump and peristaltic pump at 10 times the normal sampling flow rate.

6. Starting Sampling

Slowly insert probe through a guide cannula. **Maintain the high flow rate**. This helps to prevent the probe from clogging with the tissue debris or blood clot. Continue to flush the system at the 10 times high flow rate for 1-2 hrs. Start collection by switching back to the lower sampling flow rate such as **1 $\mu\text{L}/\text{min}$ which equals to 0020** of the flow rate setting on the ERP-10. It may be necessary to refill the Gastight Syringe with aCSF/0.15%BSA. Remember to stop the peristaltic pump at the same time or immediately after you stop the syringe pump. Alternatively you can use Eicom syringe selector, SI-60.

7. Maintenance

After the sampling: **Rinse the line with 1/100 bleach (chlorine) and let it sit for 15 minutes**. Then flush the line thoroughly with pure water. Change Peristaltic Tubing RT-5S to new one every a few sampling.

8. In Vitro Recovery Test

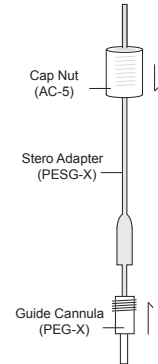
We highly recommend to check the actual flow rate of the both pumps, peristaltic pump ERP-10 and syringe pump before starting in vitro test. Please adjust the flow rate matches at least in the $\pm 5\%$ range between in and out flow. The higher flow rate of the ERP-10 results in the directly aspiration of the test samples from outside of the membrane. This is because the solution in the vials is more easily comes in the line than air from the vent. However, this problem hardly happen when you apply AtmosLM to the brain.

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.

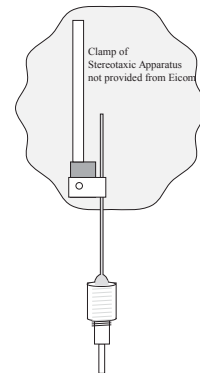
Nut.



2. Clamp the PESG-X

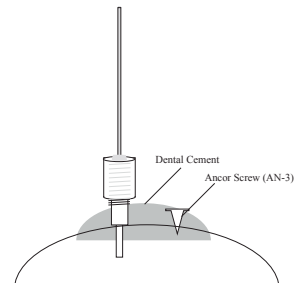
Please use standard electrode clamp which is provided from your stereotaxic apparatus manufacturer. Eicom does not carry this part.

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.

