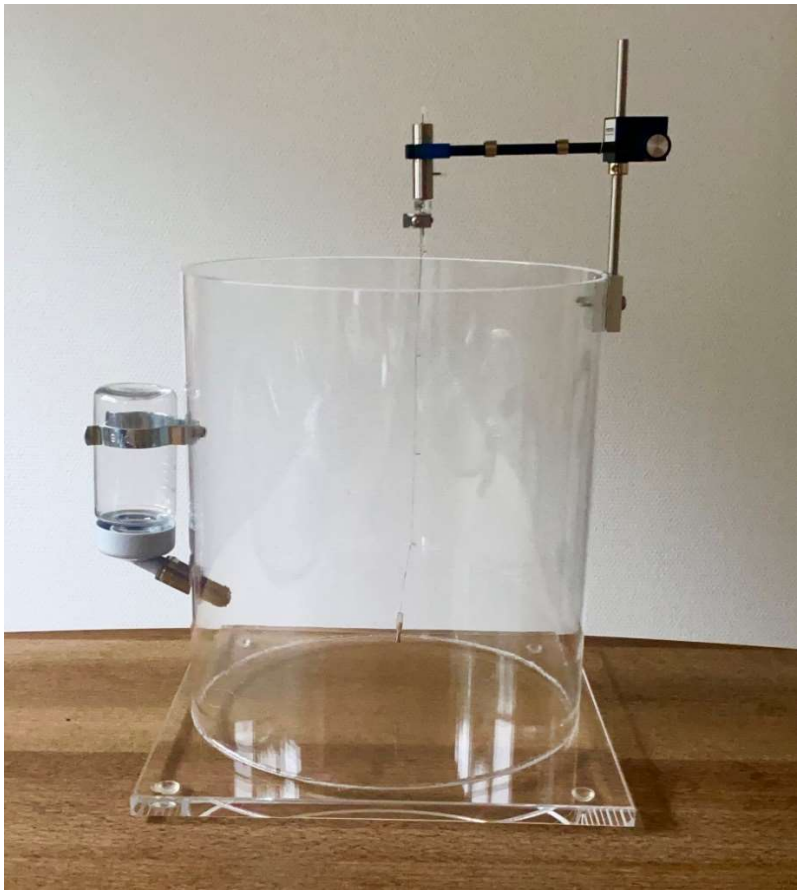
CMA Mouse Cage Manual



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1. INTRODUCTION

The CMA Mouse Cage is designed for microdialysis in conscious, freely moving mice. The cage, as part of a larger microdialysis system, allows parallel studies of animal behavior and sampling of biochemistry.

The CMA Mouse Cage is available in two configurations:

8012600 CMA Mouse Cage is equipped with Water Bottle.

8012650 CMA Mouse Cage CNS Bundle is equipped with Water Bottle, Swivel for Mouse, Multi-Axis Counter Balanced Arm, Head Block Tether for Mice, FEP Tubing (10 x 1 meter) and Tubing Adapters (10 pcs).

A complete microdialysis system for freely moving mice consists of several key components. These are application dependent and include elements for:

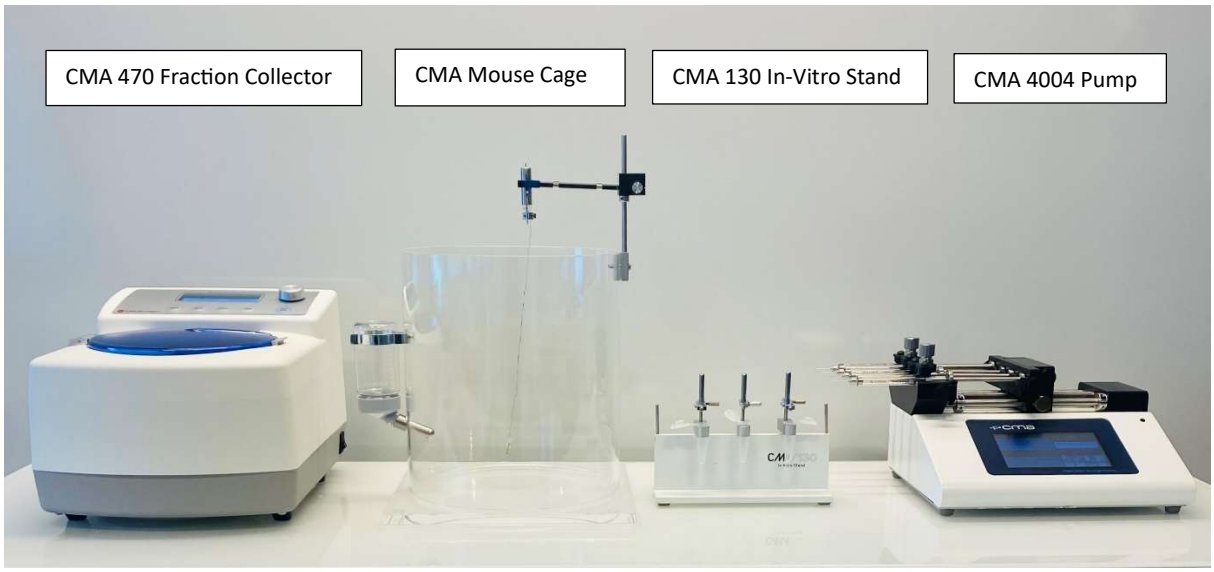
- Fluid Control: Syringe Pump, Microsyringe(s), CMA110 liquid switch, Swivel System, Peristaltic Pump for Push-Pull Configurations,
- Probe/Guide Preparation: CMA130 In Vitro Stand, Surgical Equipment for Implantation
- Experimental Housing: CMA Mouse Cage
- Sample Collection: CMA470 Refrigerated Fraction Collector
- Consumable Use: Probes, Guides, Tubing, Tubing Adapters, Vials, Perfusion Fluid

The CMA Mouse Cage will function with different kinds of microdialysis probes such as the CMA 7 and the CMA 8 model microdialysis probes. A special guide cannula has been developed for each type of microdialysis probe. The guide cannula is implanted by using a stereotaxic instrument under anesthesia, whereafter the animal is allowed to recover from the surgery. The microdialysis probe is inserted through the guide cannula into the brain of the animal while it is conscious without any more distress to the animal than the handling.

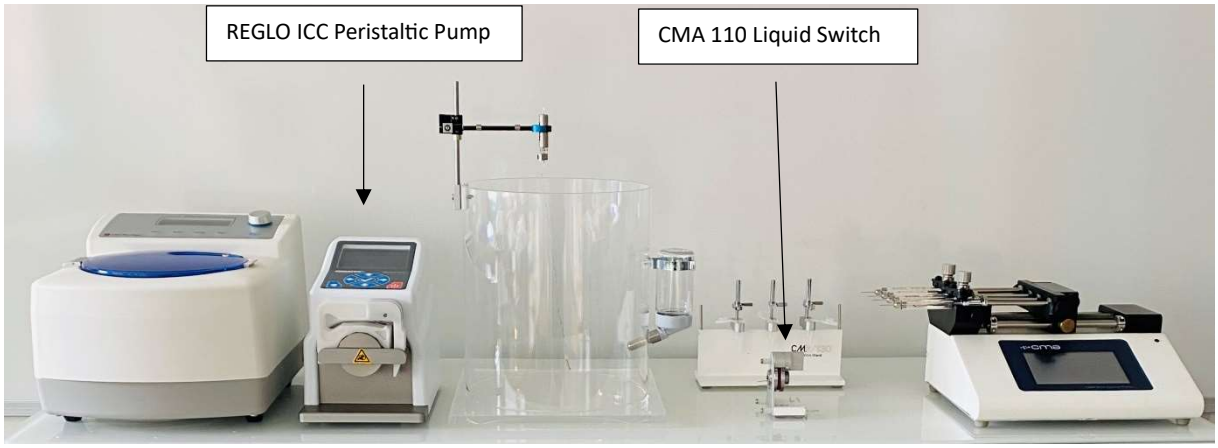
There are advantages in using a guide cannula:

- The desired target tissue for the probe remains totally undamaged
- The animal can recover fully from the surgery prior to experimentation
- The probe can be replaced as needed

Depending upon the type of investigation, an experiment can be performed directly after the insertion of the probe or the probe may be left in the brain for a period before the experiment.



Picture 1. Example of the CMA Mouse Cage Setup with CMA 4004 Pump, CMA 130 In-Vitro Stand and CMA 470 Fraction Collector



Picture 2. Example of the CMA Mouse Cage Setup with Push/Pull, CMA 4004 Pump, CMA 130 In-Vitro Stand, CMA 110 Liquid Swith, REGLO ICC Peristaltic Pump and CMA 470 Fraction Collector

2. UNPACKING

The CMA Mouse Cage Animals is delivered carefully packed to protect the equipment against damage during transportation. Save the packaging material as it provides excellent protection if it should be necessary to transport the equipment or if it is to be stored for a long period of time.

2.1 PACKING LIST

8012600 CMA Mouse Cage with Water Bottle

- Cylinder and base for Mouse Cage with pre-drilled holes for water bottle and balance arm
- Water Bottle
- Holder for water bottle
- Screw for water bottle holder
- Nut for bottle holder

8012650 CMA Mouse Cage CNS Bundle
- 8012600 CMA Mouse Cage with Water Bottle
- Swivel, Dual Channel, MD mouse
- Multi-Axis Counter-Balanced Arm
- Head Block Tether for Mice with 5 Pegs and Sleeves
- FEP Tubing 1 m 10/pkg
- Tubing Adapter 10/pkg

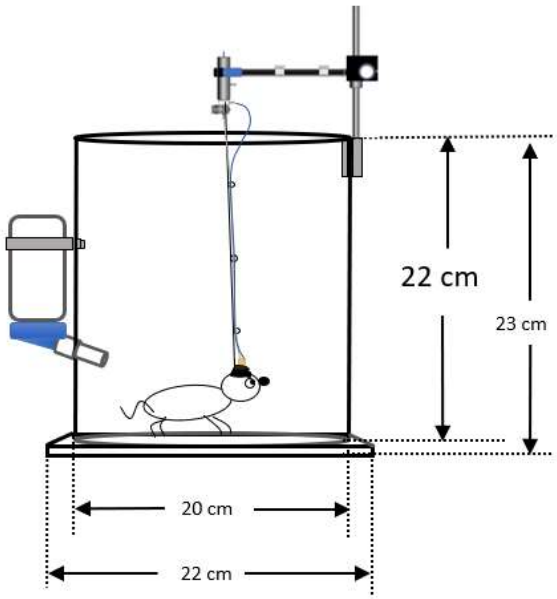
After unpacking the box, check the contents against the above packing list to ensure that the shipment is complete. Impact all items for damage or missing parts should be reported immediately to CMA Microdialysis AB or your local supplier.

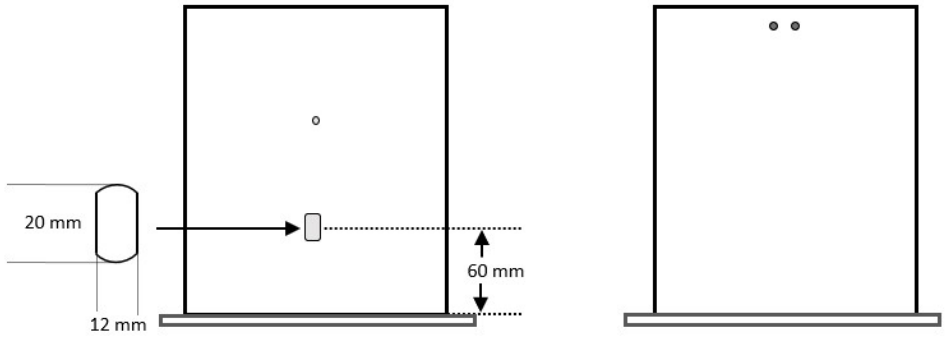
3. CAGE COMPONENTS & ASSEMBLY

The Mouse Cage is designed for small rodents such as mice or similar sized animals. The cage height is 22 cm. A short wire tether will give the animal enough torque force to turn the swivel.

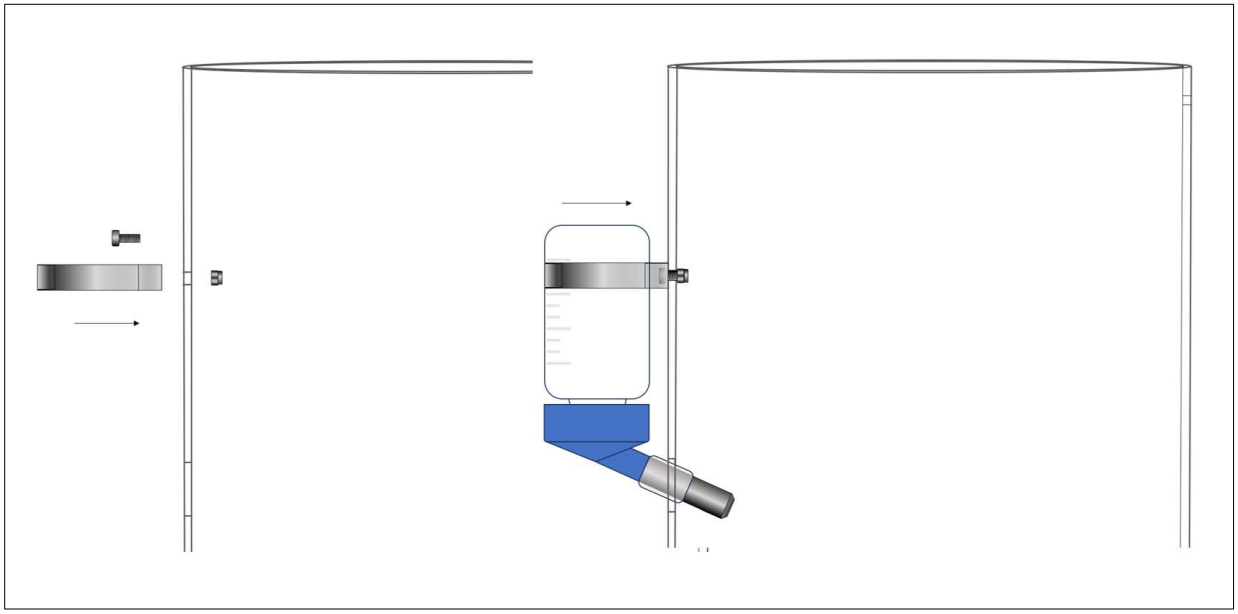
The hole for the water bottle is 6 cm above the base floor so bedding can be placed in the cage. The hole is 2 cm vertical slot, allowing the water bottle tip to be adjusted up and down a bit.

If food is needed a food bowl can be placed inside the cage.



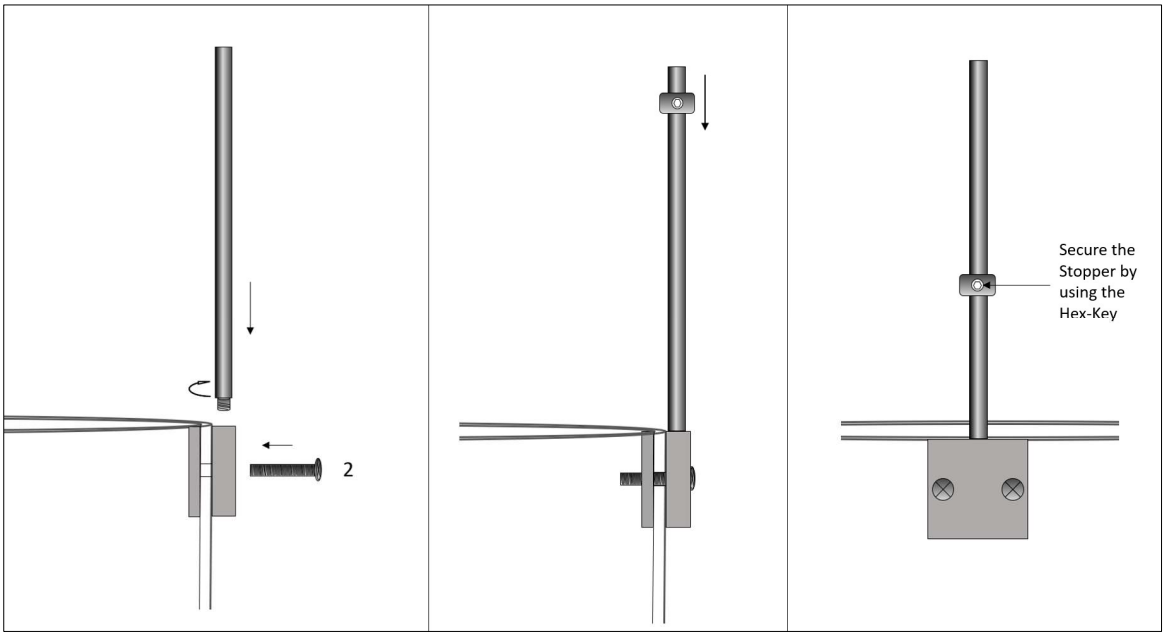


4. WATER BOTTLE



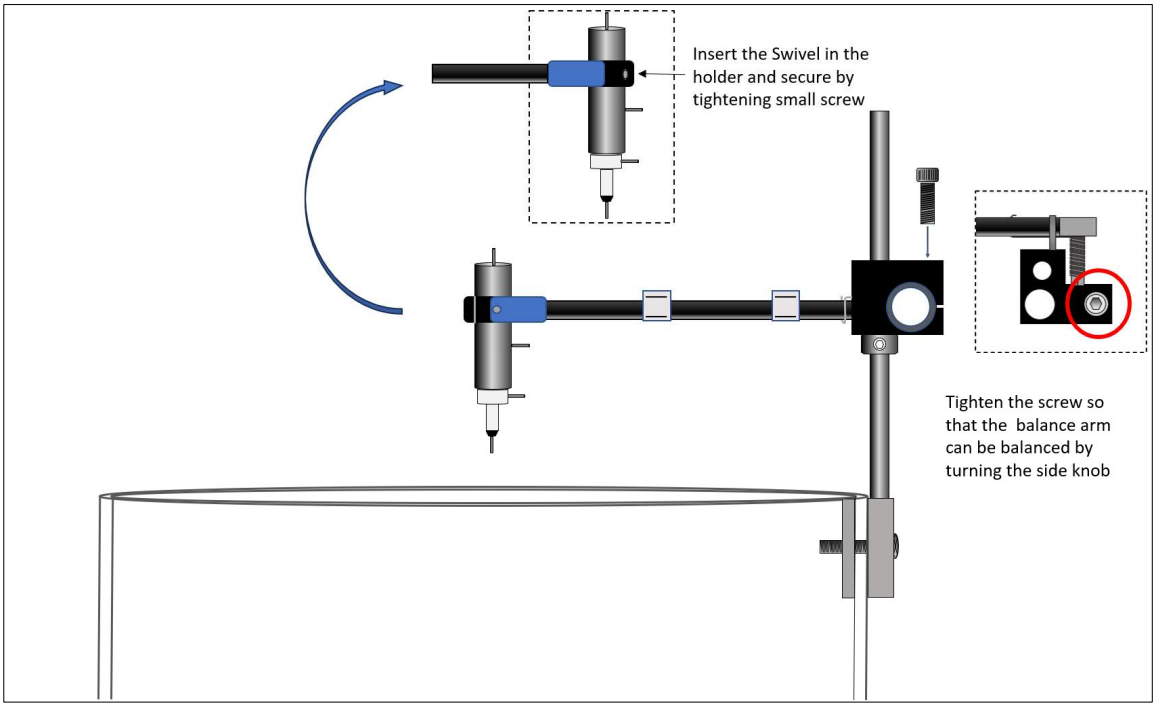
Mount the water bottle holder by using T10 Torx screwdriver and 5.5 mm wrench. The hole for the water bottle tip is 2 cm oblong shape, so you can adjust the water bottle up and down a bit.

5. MULTI-AXIS COUNTER BALANCED ARM

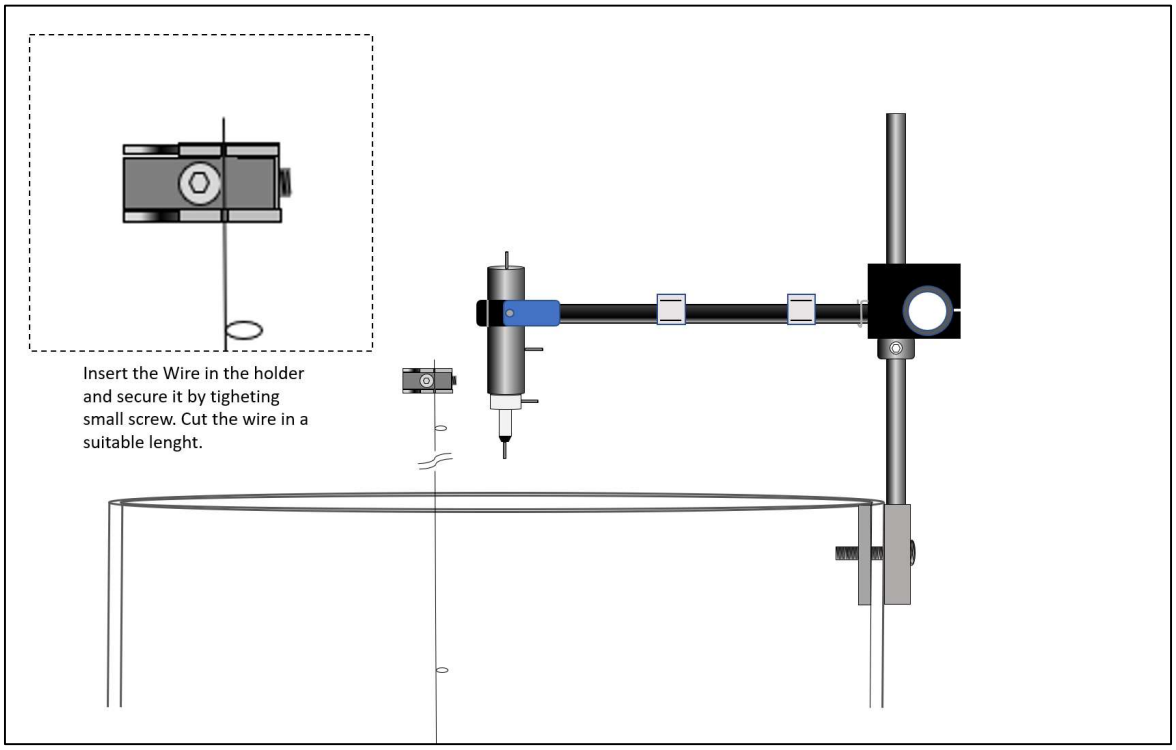


Install the Pedestal on the Cage using Mounting Plate and Screws. Slide the arm Stopper on the Pedestal and secure it on a suitable height by using the Hex-Key. **Please be careful not tightening the screws on the Mounting Plate with excess force as it may crack the Plexiglas.**

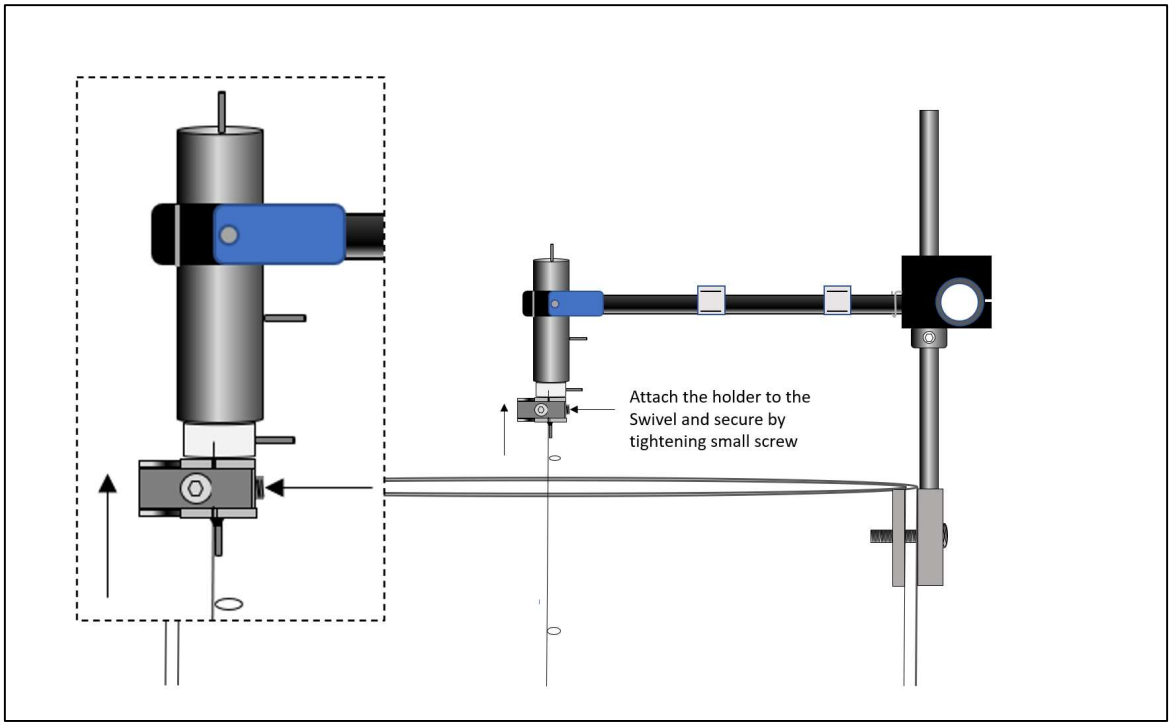
Slide the Balanced Arm on the Pedestal



Tighten the screw so the Balance Arm can be adjusted up/down by turning the side knob. Insert the Swivel in the holder and secure by tightening the small screw.

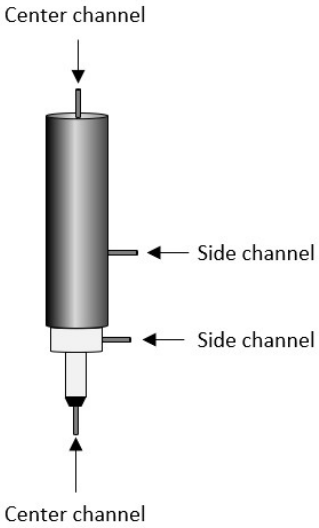


Secure the Tether Wire. On the holder, loosen the small screw that holds the plate. Insert the Wire under the plate and place the wire in the two notches. Secure the wire by tightening the small screw. Cut the wire to a suitable length.



Slide the holder on the Swivel lower tip. Secure it by tightening the side screw.

6. DUAL CHANNEL SWIVEL



The Dual Channel Swivel is a freely turning swivel with two flow channels, one straight (Center Channel) and one angled (Side Channel). The two channels have different internal volumes which is important to remember when connecting the tubing to the Dual Channel Swivel (see below). The approximate internal volumes of the Center and Side Channels are 1.8 µl and 2.8 µl, respectively.
The internal volumes can be exactly measured as follows:

- Fit a small microsyringe (25–50 µl) filled with deionized water in the Microdialysis Syringe Pump.
- Connect the inlet of the channel to the microsyringe using FEP-tubing and Tubing Adapters.
- Run the pump at a low flow rate while observing the water inside the tubing with a stereo microscope.
- When the water just reaches the inlet side of the channel, stop the pump.
- Reset the delivered volume on the pump display and restart the pump.
- Water will now enter the channel.
- Using a stereo microscope, observe the outlet of the channel and as soon as water flows from the outlet, stop the pump.
- Read the delivered volume on the pump display.
- Repeat at least three times and calculate the mean internal volume of the
- channel.

NOTE: The Dual Channel Swivel has undergone a special cleaning procedure before delivery. This prevents degradation of the substances per fused through the channels of the swivel.

7. EXPERIMENTAL PREPARATION

The CMA Mouse Cage is designed for use with the CMA 7 and CMA 8 microdialysis probes, optimized for central nervous system (CNS) applications targeting small areas of the brain or spinal cord. These probes and coordinating guide cannula feature extremely small and lightweight design ideal for use in awake mice and similarly sized spices.

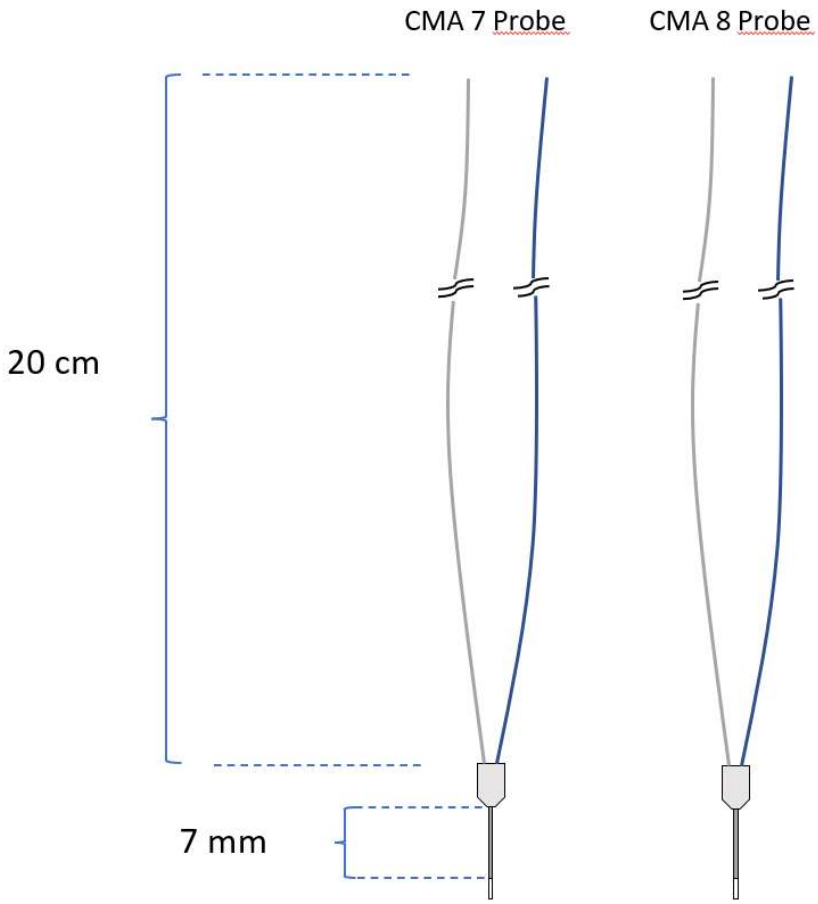
CMA´s wide range of probe options enables the user to choose the exact membrane type that suits their application, based on the physiochemical properties and size of the target molecule(s)

The CMA 7 probe is available with the following molecular weight cut-offs: 6 kDa, 55 kDa, 500 kDa and 2 MDa.

The CMA 8 probe is available in two molecular weight cutoffs: 20 kDA and 100 kDA.

For translatable studies in larger species, identical membrane options are available in for the rat CNS probe models: the CMA 11 and the CMA 12.

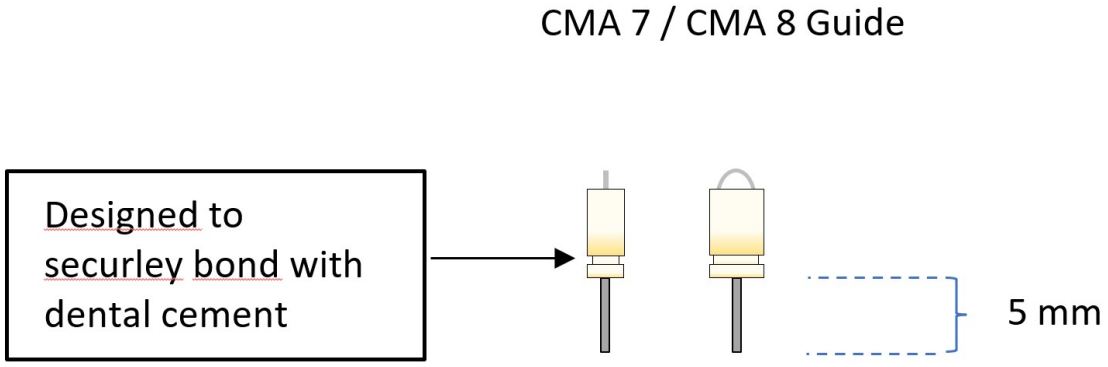
CMA 7 and CMA 8 are not intended for use in humans. The are only suitable for laboratory research in animals. CMA Microdialysis guarantees all probes for single use.



	CMA 7	CMA 8
Shaft Length	7 mm	7 mm
Shaft OD	0.4 mm	0.4 mm
Membrane Length	1 or 2 mm	1 or 2 mm
Membrane Diameter	0.24-0.28	0.5 mm
Membrane Cut-off	6 kDa	20 kDa
	55 kDa	100 kDa
	500 kDa	
	2 Mda	
Inlet Tubing (Blue)	20 mm	20 mm
Outlet Tubing (Transp.)	20 mm	20 mm

7.1 CMA 7/CMA 8 GUIDE CANNULA

The coordinating guide cannula for the microdialysis probes have been developed for chronic implantation in the brain of mice and small animals. -Both are factory-made with high precision for an exact, reproducible fit between batches. A -dummy probe, consisting of a pin with plastic holder is pre-inserted into the guide cannula to ensure patency until the time of the experiment. On the day of experiment, the dummy is removed and replaced with the microdialysis probe. The Guide Cannula for the CMA 7 and CMA 8 Microdialysis Probes are cemented directly to the skull. The guide cannula is coated with silicon on the inside to prevent the sticking of a dummy or a probe.



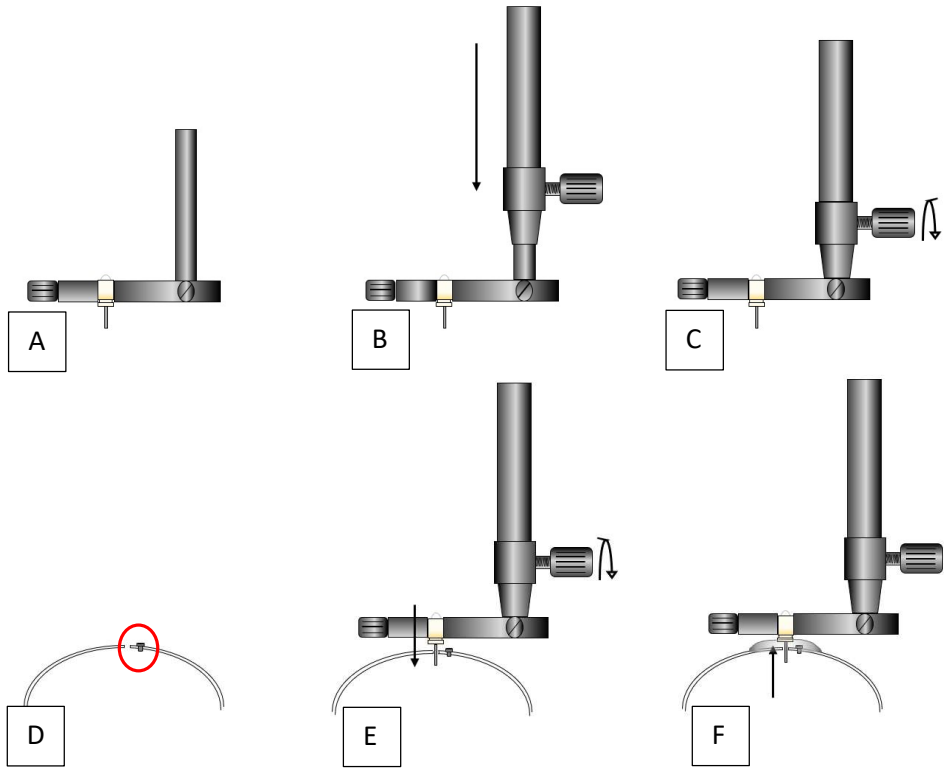
7.2 IMPLANTATION OF THE GUIDE CANNULA

In preparation for the microdialysis experiment, the guide cannula is implanted within the animal by means of stereotaxic surgery. Animals are allowed to regain consciousness and recover, per laboratory protocol, before the probe is introduced and microdialysis sampling commences.

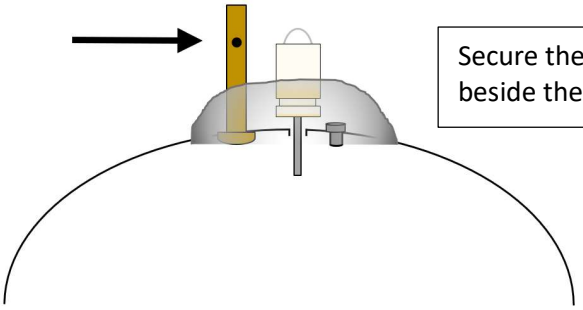
The CMA 130 In Vitro Stand is an invaluable tool for purposes of guide and probe preparation. The 8309104 version includes specialized supports to submerge 3 mouse CNS probes in 1.5 mL Eppendorf tubes to facilitate in vitro calibration protocols, pre-experimental priming, and post-experimental storage. It additionally provides a stereotaxic holder consisting of a CMA 7/8 clip, connection rod for clip, and two type of stereotaxic adapters (for KOPF, Stoelting, HB, RWD Stereotaxic Instruments).

8309104 CMA 130 In-Vitro Stand with CMA 7/8 Clips

The steps below describe use of the CMA130 stereotaxic holder for placement of the guide cannula.

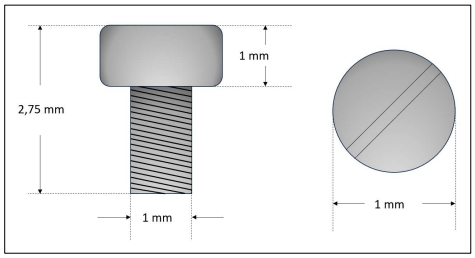


A: Mount the Guide Cannula in the CMA 7/8 Clip. **B and C:** Fix the Clip to the Connecting Rod and connect it to the Manipulator Arm on the Stereotaxic instrument. **D:** Make a burr hole in the skull using a 0.6 Ø Drill Bit. Be careful to not drill too far. Drill holes for the Anchor Screws by using a 0.9 Ø Anchor Screw Drill Bit. **E:** Lower the Guide through the hole and meninges. There is no need to open the meninges before inserting the Guide. Insert the Guide to the point where the guide tip reaches start of your target area. **F:** Secure the Guide with Dental Cement.



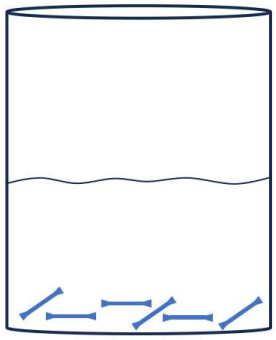
Secure the Peg beside the guide

It is essential that the guide and peg are properly secured. Several anchor screws may be needed.



CMA7431021, Anchor Screws, 100/pkg
CMA8003264, Anchor Screw Drill Bits, 3/pkg
CMA8309673, Screw Driver for Anchor Screws
620020, Carbide Drill Bit, Round, 0.6 mm 5/pkg

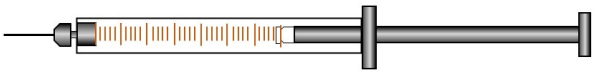
7.3 TUBING CONNECTIONS



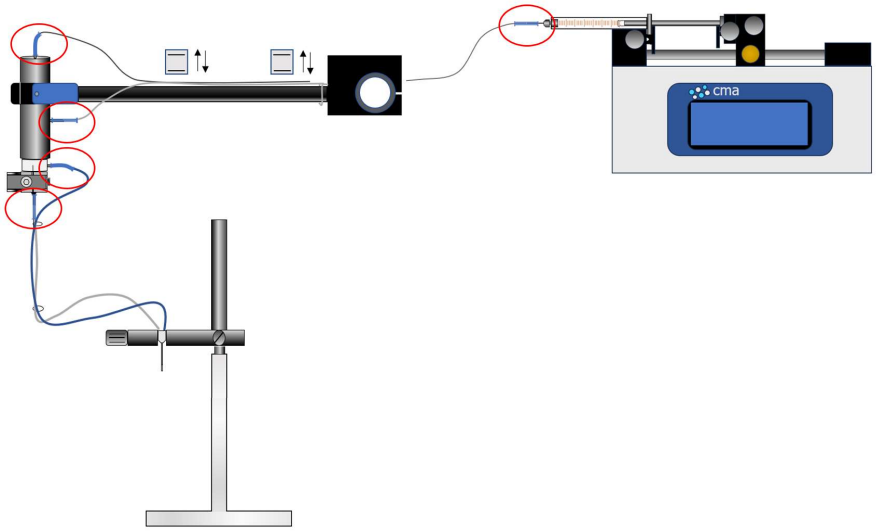
70% Ethanol

Tubing Adapters

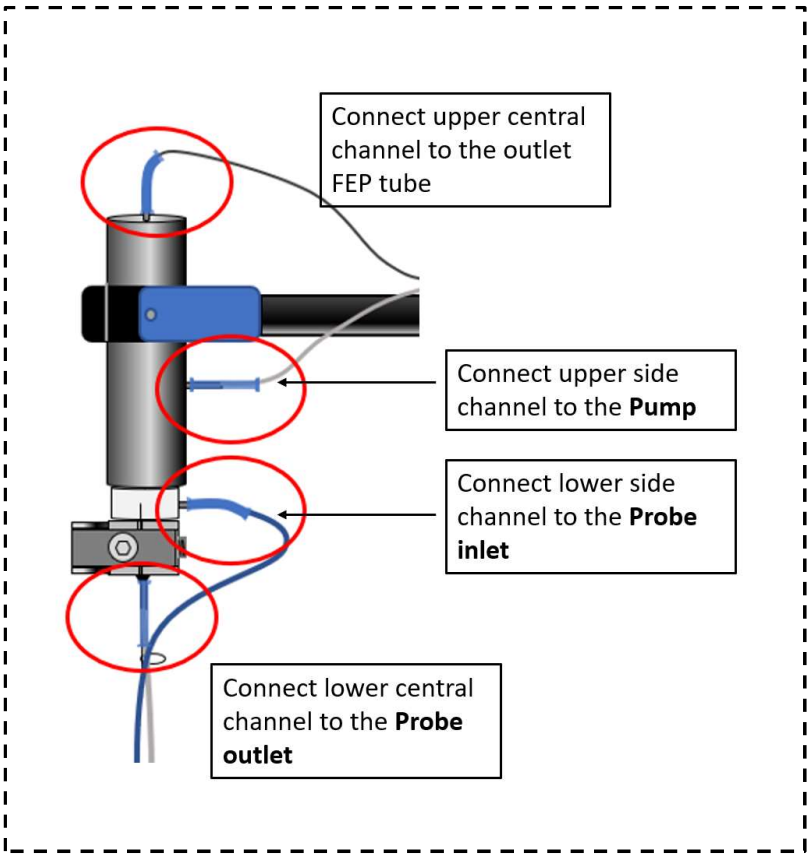
Soak the Tubing Adapters into 70% Ethanol for 1 hour. The Tubing Adapters swell in 70% Ethanol and shrink back in air, ensure tight, zero internal volume connections between FEP Tubing and the probe, swivel liquid switch and syringes. Tubing adapters can be left in Ethanol for a long time (months), so you always have tubing adapters ready for use.



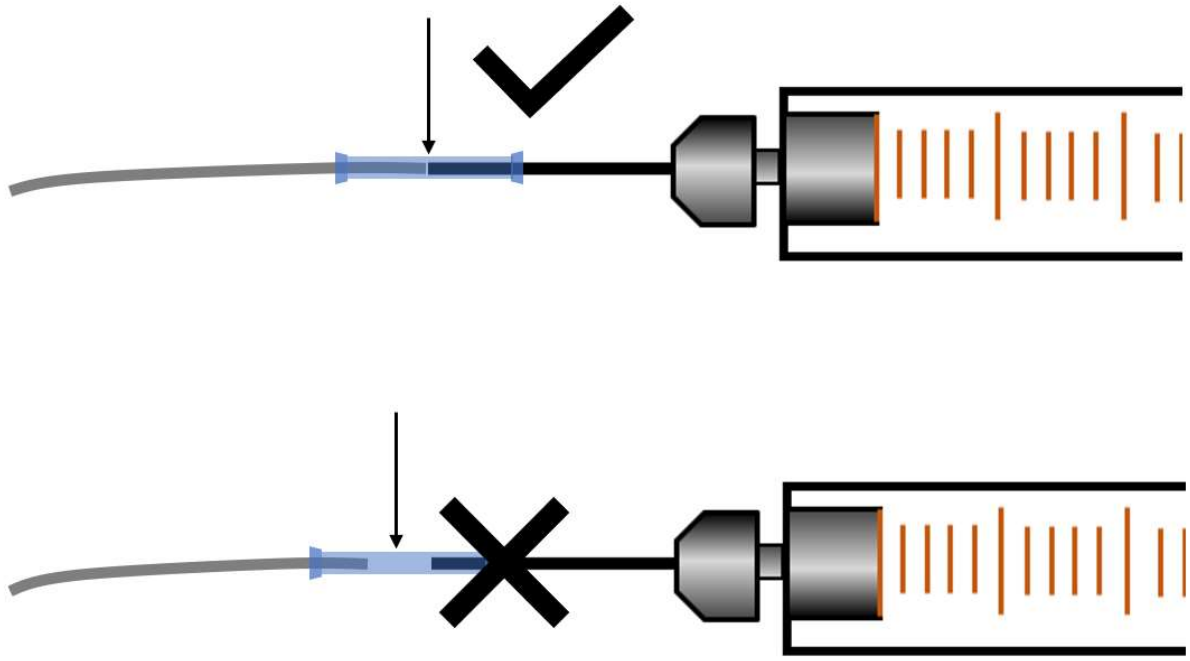
Fill a Syringe with Perfusion Fluid. The Perfusion Fluid should be at **room temperature** or slightly higher. This makes it easier to remove air bubbles and prevents air bubbles being formed during the experiment. Make sure that even the smallest air bubbles are gone.



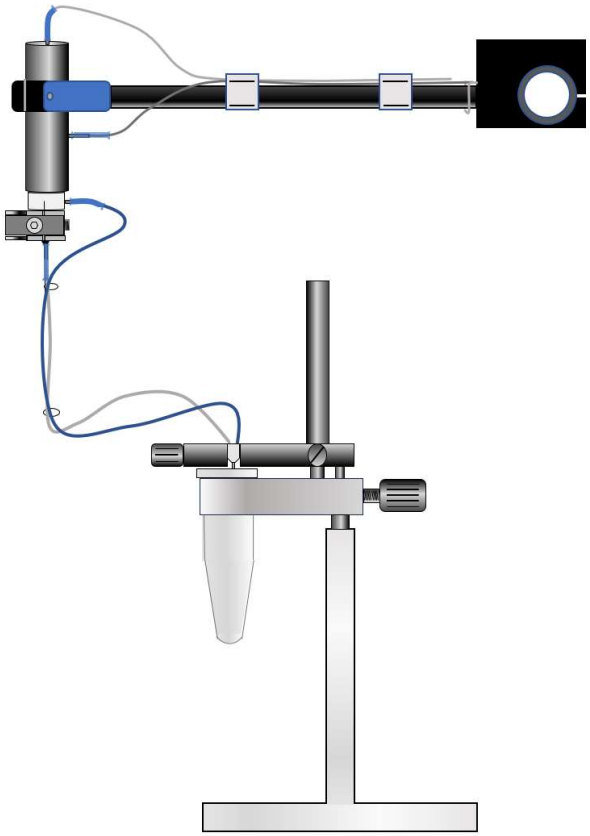
Mount the Microdialysis probe in the CMA 7/8 Probe Clip. See also CMA 130 Manual. Connect suitable length of FEP Tubing to the Syringe and Swivel with Tubing Adapters. Secure the FEP tubing on the arm. Connect the Inlet FEP tube to the Syringe Tip and to the upper side channel of the Swivel. Connect the Blue Inlet to the lower side channel. The transparent outlet tube to the lower center channel. The upper center channel is connected to the outlet FEP Tube. The outlet FEP tube can be connected to a fraction collector or to a stand-alone vial for -manual collection. The outlet tube should be as short as possible to prevent unnecessary back pressure.



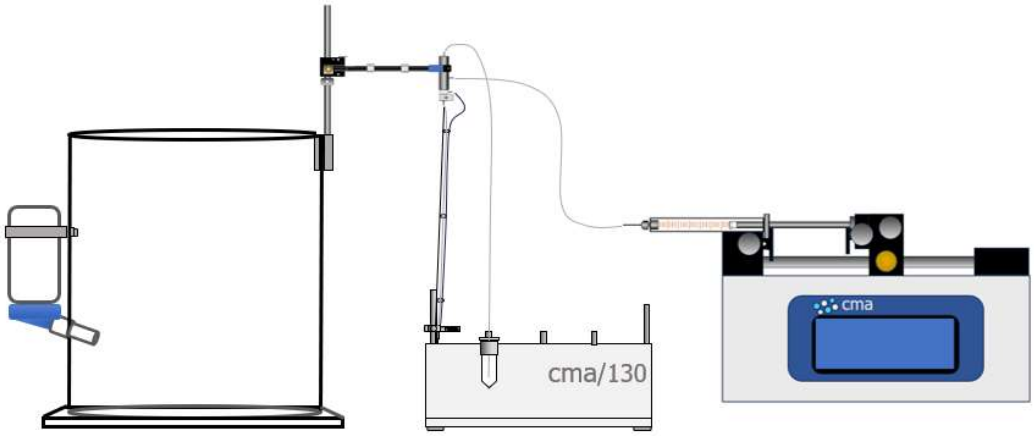
Ensure the ends meet for zero dead volume and that the tubing adapter is not exposed to the perfusion fluid. Once the Tubing Adapters are dry, they form of a tight, leak free, connection.



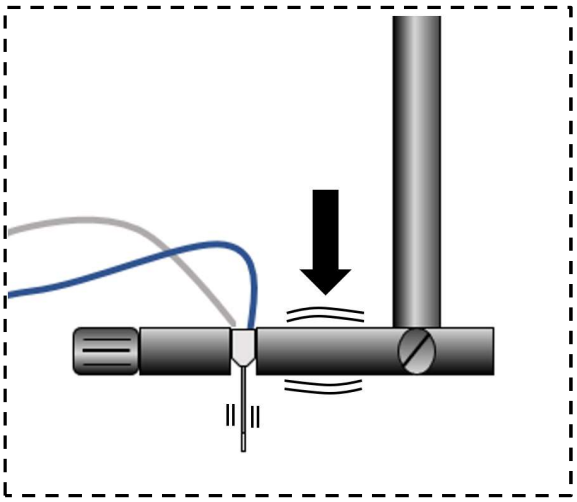
7.4 PRIMING THE TUBING, SWIVEL AND PROBE



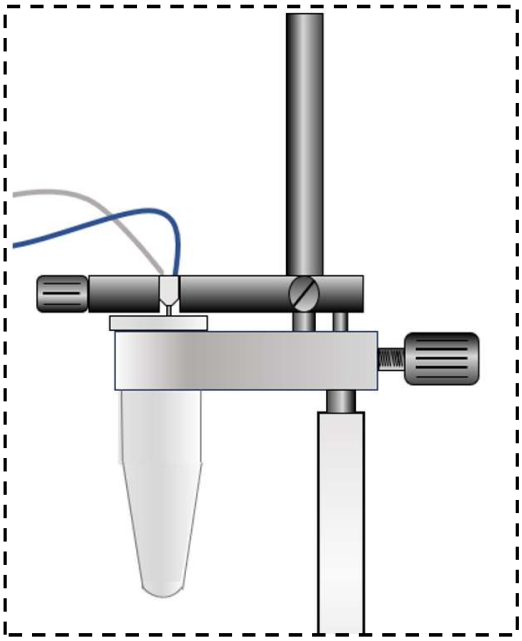
Submerge the probe in a vial filled with Perfusion Fluid on the CMA 130 In-Vitro Stand.



Prime the probe by flushing the tubing, swivel and probe with a flow rate of 10-15 µl/min and wait until fluid exits the outlet tubing. When you observe fluid from the outlet, lift the Probe Clip of the In-Vitro Stand and hold the Clip with your hand. Do not stop the flow.



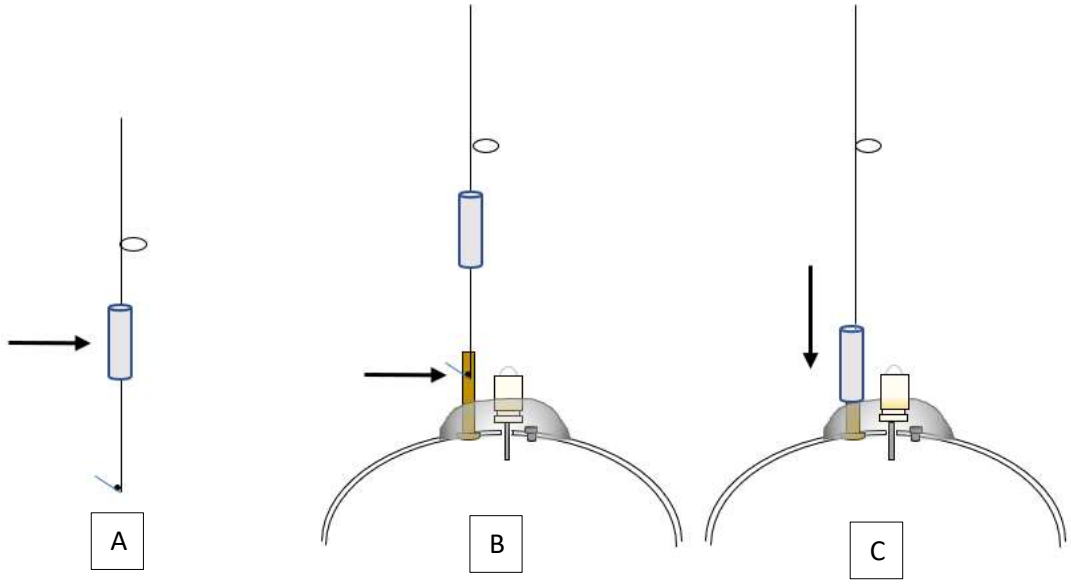
Priming the probe and tubing with Perfusion Fluid:
Raise the Probe Clip. Flush the probe with 10-15 µl/min for 4-5 min. While flushing, “tap” gently with a scissor (or other metal implement) on the probe clip to create vibrations. The vibrations from the probe clip remove air bubbles under the membrane.



Priming the probe and tubing with Perfusion Fluid:
After flushing is complete place the probe back into the perfusion fluid in the In-Vitro stand. Continuously perfuse the probe with 0.3 µl/min until it is time to insert the probe into the guide. It is important that no salt crystals are formed inside that can block the probe and tubing.

7.5 SECURE THE TETHER

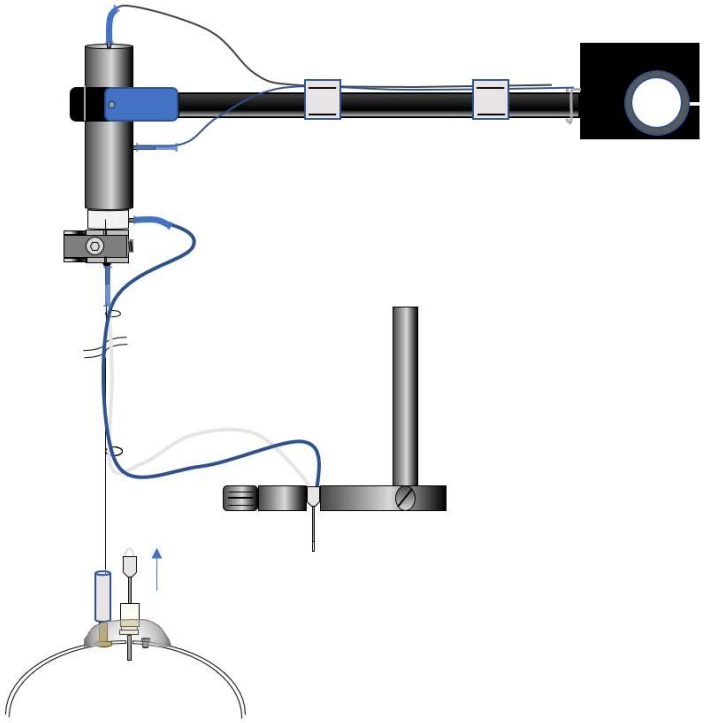
Secure the wire to the peg. Hold the mice still by gripping the neck and gently push the mice against a flat surface. Follow the procedure below.

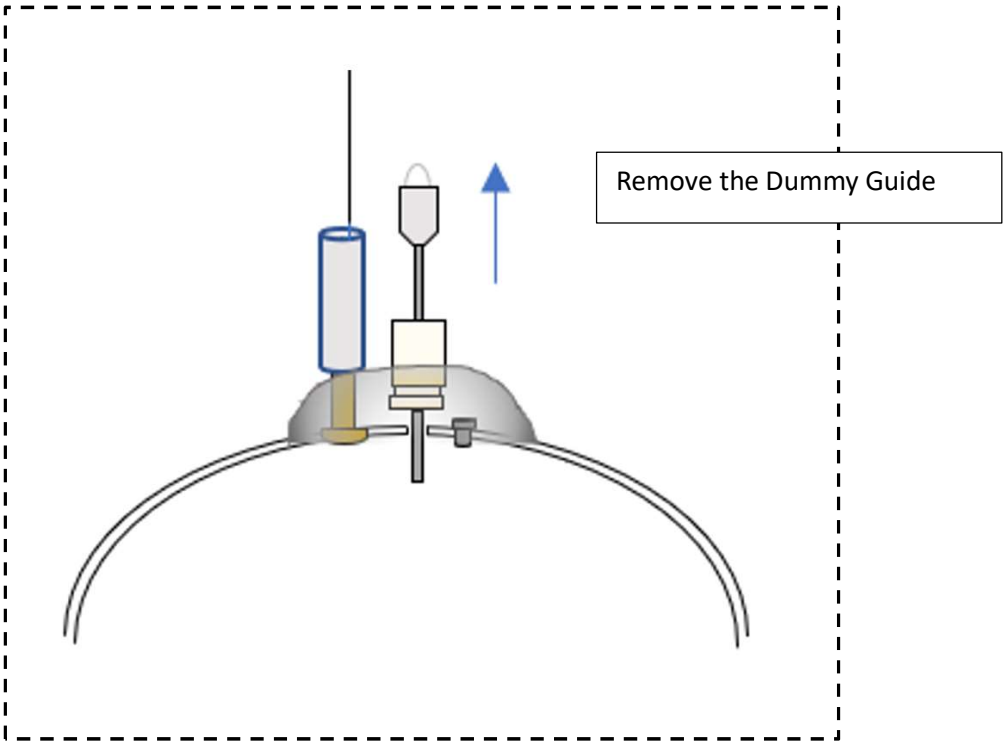


A. First slide the silicone tube on the wire. **B.** Insert the distal end of the wire into the hole on the Peg. **C.** Secure the wire by sliding the silicone tube on the Peg.

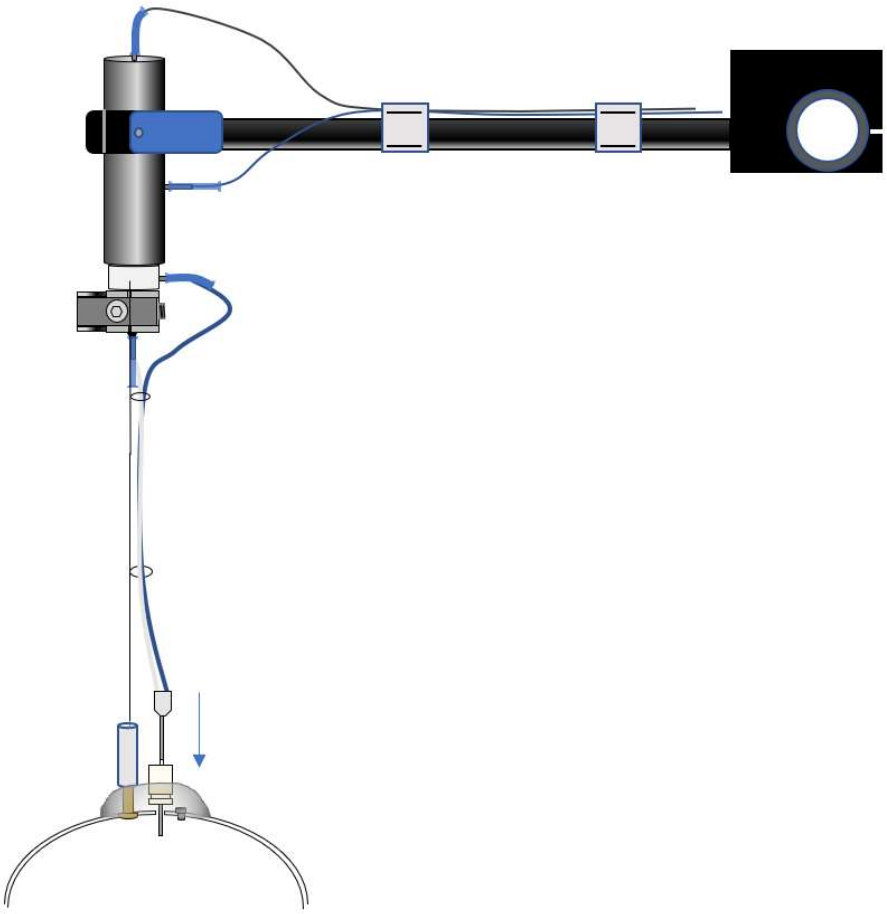
7.6 INSERTION OF THE PROBE

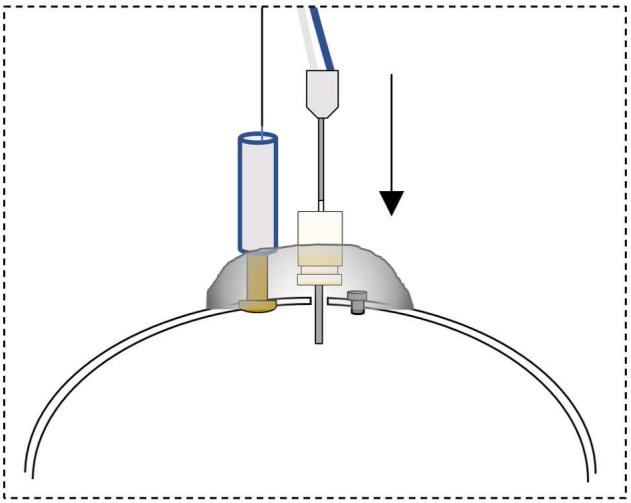
Use the same procedure as above. Hold the mice still by gripping the neck and gently push the mice against a flat surface. Remove the dummy from the guide and fully insert the probe into the guide so the membrane is exposed in the tissue.

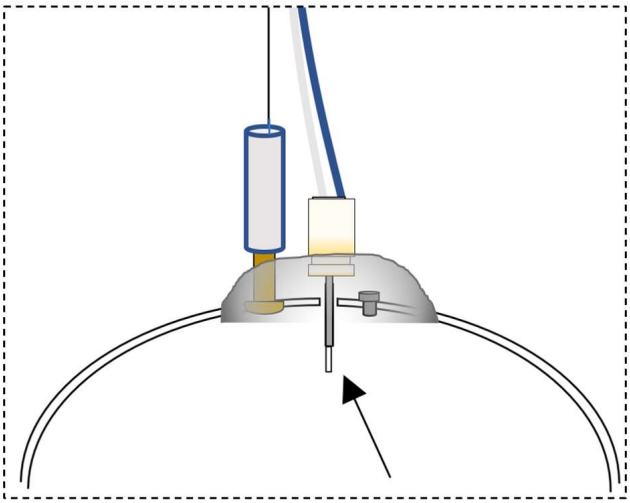




Fully insert the Probe into the Guide so the membrane is exposed in the tissue.







7.7 INSERTION OF THE MICRODIALYSIS PROBE (6 kDa and 20 kDa membranes) INTO THE GUIDE CANNULA WHEN THE PROBE IS NOT TO BE PERFUSED IMMEDIATELY

1. Implant the Guide Cannula as described above and allow the animal to recover.

2. Prepare and perfuse the Microdialysis Probe according to the manual.
Insert the probe through the Guide Cannula and secure it while continuously perfusing the probe.

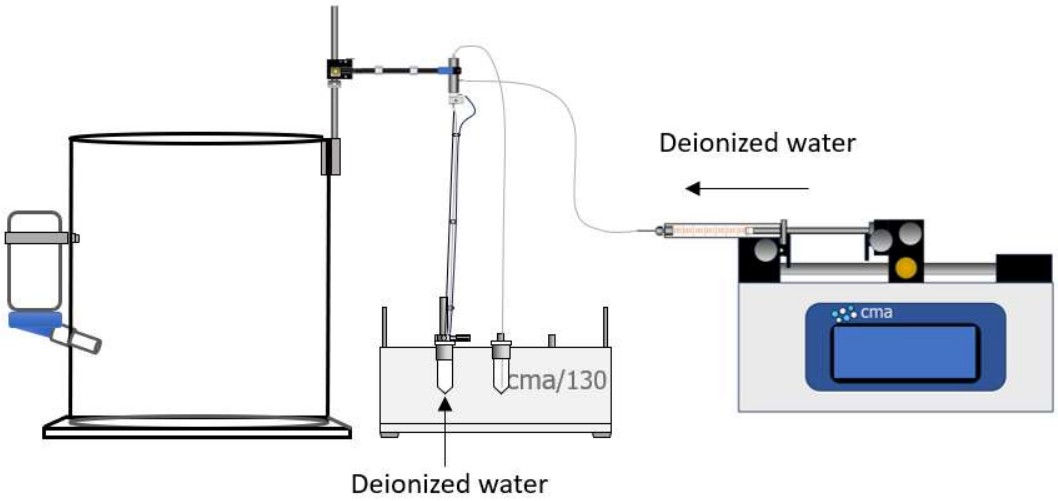
3. Cut the inlet (do not use a scissor. Use a sharp knife and cut the tube in a sliding movement) and outlet tubing of the probe to approximately 3 cm length. Connect the two tubing together by the use of a Tubing Adapter.

4. Return the animal to its cage.

5. When the experiment is to begin, cut the distal ends of the inlet and outlet tubing of the probe to remove the Tubing Adapter and make sure that liquid fills the tubes completely. Attach appropriate lengths of FEP-tubing to the swivel and to the vial collecting stand. Perfuse the swivel and its channel to make sure that there is no air trapped in the system. It is particularly important that the Tubing Adapters are filled with perfusion liquid to prevent air bubbles entering the probe.
Connect the animal to the swivel. (Two people may be required, one to hold the animal, and one to connect the tubing.)

Please note that above may not work with high cut-off and ultra high cut-off probes as larger molecules will pass over the membrane and block the tubing.

7.8 RE-USING THE PROBE



If re-using the probe. It is important to thoroughly clean the probes to prevent formation of salt deposits when attempting to reuse post-experiment. To prepare for storage, fill a syringe with deionized water and submerge the membrane in deionized water using the In Vitro Stand. Flush the probe at a flow rate of 10 µl/min for 30-60 minutes. The probe can be stored wetted in deionized water using the In Vitro Stand. Alternatively, the probe can be stored dry in the In Vitro Stand by using a syringe in withdrawal mode to flush air through the swivel channel, tubing, and the probe.

Please note that probes are consumable and guaranteed by CMA for single use. Repeated use of the probes may decrease functionality. An In Vitro calibration test with standards can be implemented to assess performance prior to reuse.

Do not use alcohol for cleaning the probe!

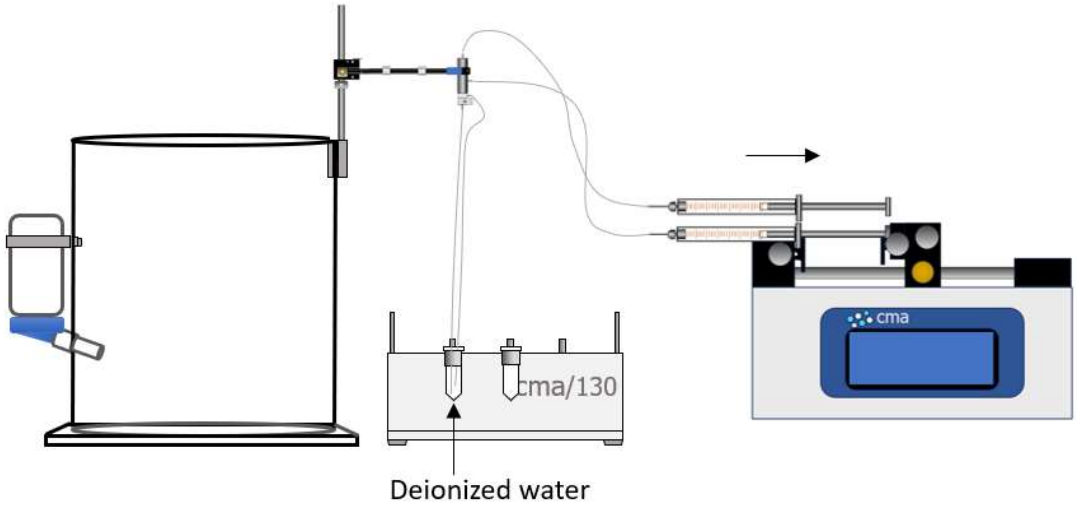
8. MAINTENANCE

8.1 CLEANING THE CAGE

The cage can be cleaned with soap and water. For disinfection use Cidex_OPA or Mucasol or Hydrogen Peroxide (H2O2) Gas Plasma Sterilization. Do not use alcohol or autoclaving as it will damage the Plexiglas.

8.2 CLEANING THE DUAL CHANNEL SWIVEL

It is essential that the channels of the swivel are cleaned after use. If this is not done, salt crystals from the perfusion fluid may form, which can block and seriously damage the channels.
NOTE: Always clean the channels of the swivel after use by pulling deionized water through the swivel. Pull air through the channels. Keep dry.



After the experiment, remove the probe and attach FEP tubing. Put the FEP tubing distal ends into a vial filled with deionized water. Load the pump with 2 empty syringes and attach to the Swivel with FEP tubings. Pull (aspirate) with 10 µl/min for 30-60 minutes.

8.3 CRYSTAL-BLOCK OR LIQUID LEAKAGE DUAL CHANNEL SWIVEL

If the channels are blocked by salt crystals or begin to leak, replacement of the swivel with a new unit is recommended. Alternatively, the Swivel may be sent back to the manufacturer for repair. Please contact CMA or your local supplier.

9. STORAGE

If the CMA Mouse Cage is not to be used for a significant length of time:
- clean all part of system.
- store system in shipping carton in safe place.

The above suggestions will help keep your CMA Mouse Cage running smoothly and in good condition.

For advice, service or technical assistance, contact your local supplier or CMA Microdialysis AB.

10. TECHNICAL DATA

10.1 CAGE

Height: 220 mm
Diameter: 200 mm
Material: Perspex Acrylic

10.2 DUAL CHANNEL SWIVEL

Housing dimensions: 9.5 x 33 mm

Internal volumes: Centre Channel = 1.4 µl

Side Channel = 2.8 µl

10.3 SERVICE

CMA and CMA distributors have skilled service staff to solve your technical problems if an equipment-oriented problem should arise.

Europe:
E-mail: cma@microdialysis.se
Phone: +46 8 470 1000
Web: www.microdialysis.se

US:
E-mail: support@hbiosci.com
Phone: 800-547-6766
Web: www.harvardapparatus.com

World:
Please contact your local supplier.

11. ORDERING INFORMATION

Name	Ref. No.
CMA Mouse Cage	CMA 8012600
CMA Mouse Cage CNS Bundle	CMA 8012650
CMA 4004 Syringe Pump	CMA 400400
CMA 402 Syringe Pump	CMA 8003110
CMA 402 Syringe Pump with Accessory Kit	CMA 8003100
CMA 470 Refrigerated Fraction Collector	CMA 8002770
Microsyringe 1 mL, glass	CMA 8309020
Microsyringe 2.5 mL, glass	CMA 8309021
Microsyringe 5 mL, glass	CMA 8309022
Micro-T	CMA P000043
Perfusion Fluid CNS, 5 mL x 10 amp.	CMA P000151
Artificial CSF, 25 mL x 6 vials	597316
Tubing Adapters 10 pcs	CMA 3409500
FEP Tubing 1 meter	CMA 3409501
FEP Tubing 1 meter x 10	CMA 8409501
Dual Channel Swivel for Mouse	720000
Head Block Tether (incl 5 pegs, 5 sleeves)	610037
Multi-Axis Counter-Balanced Arm	610024
Anchor Screws, 100 pcs	CMA 7431021
Anchor Screw Drill Bits, 2 pcs	CMA 8011158
Screwdriver for anchor screws	CMA 8309673
Carbide Drill Bit, Round, 0.6 mm, 5 pcs	620020
CMA 130 In-Vitro Stand with CMA 7/8 Clips x 3	CMA 8309104

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