

BR Brain Probes User's Guide

NOT APPROVED FOR USE IN HUMANS! THESE PRODUCTS ARE DESIGNED SOLELY FOR USE IN EXPERIMENTAL ANIMALS.
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Introduction

A new microdialysis probe must be prepared prior to beginning either an *in vitro* recovery experiment or *in vivo* sampling study. Some of these steps should also be repeated when reusing a microdialysis probe. Although BR probes are not warranted for more than a single use, they may be used again until the membrane is no longer viable or another probe component is damaged.

The Dialysis Membrane

The membrane in the BR-2, BR-4, or custom BR probe is filled with microscopic pores. During the dialysis experiment, the analyte will diffuse through these pores into the probe. A drug may also be simultaneously delivered by the probe, diffusing through the pores and into the tissue being studied. The pores in new probe membranes are filled with glycerol. The glycerol keeps the pores open and the membrane moist. It must be flushed out prior to a microdialysis experiment.

Under no circumstances should the probe membrane be allowed to dry after it has once been wetted. Keep the membrane tip immersed in fluid (water or perfusion fluid). The shipping vial provides a safe means of long-term storage of wetted probes. The *Calibration Station* accessory (MD-1522) is useful for short-term storage, probe preparation, and *in vitro* recovery studies.

Probe Preparation

Preparation of probes involves the following steps:

Wet Probe. DO NOT SOAK PROBES IN ALCOHOL.

New probes are shipped dry and must be flushed and wetted thoroughly. Previously used probes must be kept wet at all times to remain viable.

Eliminate Air Trapped air (bubbles) must be purged from inside the probe and all connecting tubing and cannulae.

Check for Leaks. Examine all connecting tubing, connectors, syringe needles, etc. to look for any sign of leaking caused by improper or inappropriate connections.

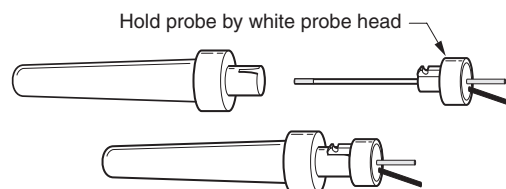
Procedure

1. Fill a gastight microdialysis syringe (e.g., BASi Bee Stinger, 500 mL) with filtered (0.2 mm membrane filter, regenerated cellulose), degassed perfusion fluid at room temperature. Mount the syringe in the pump.

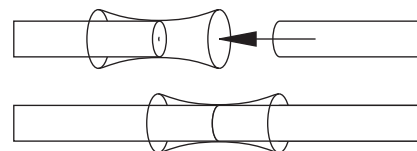
Air bubbles will form in the syringe and connecting tubing lines when they are filled with cold perfusion fluid. Warm fluid to room temperature before use.

2. Start the pump and make sure that fluid appears at the tip of the syringe needle. Stop the pump.
3. Open the probe package by placing the clear plastic side of the tray down. Tear away the package seal by starting at the corner indicated. **Probes are delicate instruments! Exercise extreme care whenever handling a probe.** Carefully invert the opened tray onto your other hand to catch the probe, or reach into the tray to grasp the probe shipping vial and pull it straight up. Don't tilt or pivot the vial against the tray since this may damage exposed inlet and outlet tubes.

4. Holding the shipping vial with one hand and the white probe head (not the inlet or outlet cannulae!) with the other, gently pull the probe straight out of the vial.



5. Carefully insert the probe in the U-shaped cavity on the Probe Clamp (MD-1522). Position the probe so that the head's "key" faces the open end of the U cavity. Slowly tighten the clamp knob. The probe head should self-align in the clamp.
6. Flanged tubing connectors (MD-1510) and FEP Teflon® Tubing (MF-5164) should be used for all connections. Cut desired lengths of tubing using a razor blade. Make a clean, blunt cut. **DO NOT USE SCISSORS** to cut tubing. Insert tubing halfway into a fresh connector.



Flanged tubing connectors join FEP tubing to inlet and outlet cannulae. Tubing should touch the cannula being joined, leaving no dead space in between.

7. Slide connectors and tubing onto the syringe and fill the tubing with perfusate. The syringe needle should abut the tubing with no dead space in the connection.

YELLOW = INLET Cannula
GREEN = OUTLET Cannula

8. Using connectors, connect the other end of the FEP tubing to the microdialysis probe inlet (yellow). Again, avoid any dead space in the connection. If so equipped, do not plumb the white, Teflon-lined liquid swivel yet. To maximize flush rates, you may choose to not connect outlet tubing until after step 13.
9. Fill a vial on the other side of the Calibration Station with the desired perfusion fluid (artificial CSF, Ringer's solution, etc.) or clean, distilled water. **WARNING! Do NOT soak probes in alcohol.**
10. Using the clamp, transfer the probe to the vial of fluid in the Calibration Station. The membrane tip should now be immersed in fluid. **If you flush the probe while using the white, Teflon-lined liquid swivel, do not perfuse at flow rates > 5 mL/min. Disconnect the outlet tubing between the probe and swivel prior to flushing.**

11. Prior to flushing, first disconnect the probe outlet from any liquid swivels or other devices that are attached. Set the pump flow rate according to the amount of tubing remaining on the probe outlet (see chart).

Max. Flow Rate (mL/min)	Outlet Tubing Length (mm)
25	0 - 100
20	200
10	300

12. Check for trapped air in the membrane tip by examining it with a magnifying lens or dissecting microscope. Keep the probe in the probe clamp. Always maintain flow through the probe so that the membrane remains moistened. Trapped air shows a meniscus between the liquid and air surfaces. If flushing doesn't expel the air, return the probe and clamp to the Calibration Station, then rap the Station on the table top. Do not hit or flick the probe itself.

At elevated flow rates, the membrane may appear to "sweat." This is not leaking. It is ultrafiltration of perfusion fluid through membrane pores, which may occur if the perfusion flow rate is high. It can also be caused by excessive back pressure on the probe from long lengths of outlet tubing, crimped or clogged tubing, swivel blockage, or failure of another system component located downstream from the probe. This can be confirmed by monitoring flow.

13. When the probe lumen is bubble-free, return the probe to the Calibration Station and flush for 5-10 additional minutes (per recommendations above) to clear residual glycerol from the membrane.
14. Now the probe is ready to use. Set the pump to the final flow rate (usually 1 to 5 μ L/min). Connect all lines to swivels, fraction collectors, on-line injectors, etc. Start the perfusion again and check for leaks. Remove the probe from the probe clamp prior to insertion into an intracerebral guide cannula. Remember, keep the probe membrane in solution (being perfused) and/or in tissue.
15. For anesthetized animal microdialysis, use the MD-1521 Clamp Rod to hold the clamped probe in your stereotaxic frame. For awake animal microdialysis, consult the separate User's Guide included with BASi intracerebral guides for instructions.

Storage

When the experiment is complete, mount the probe in the probe clamp and lower it into a water-filled vial on the Calibration Station. Use clean, distilled water. Thoroughly flush the probe and all connecting tubing and accessories at normal flow rates (1-5 mL/min) to eliminate all residual salts in the system. Between uses, store probes in distilled water in the Calibration Station for short periods of time. For longer storage, store probes in the

original shipping vial using a 0.005% (final concentration) ProClin150™ preservative to retard bacterial growth. Wrap the top of the vial with laboratory film to prevent evaporation. ProClin150 is sold by BASi (CF-2150) and must be diluted prior to use. Be sure to thoroughly flush the probe with water before reuse.

Ordering Information

- MD-2200 BR-2 Brain Microdialysis Probe, 2 mm membrane, 6/pkg.
- MD-2204 BR-4 Brain Microdialysis Probe, 4 mm membrane, 6/pkg.
- CUSTOM Any other membrane length, no additional charge and up to 6 weeks delivery time, 6/pkg.
- CUSTOM Any other cannula length, 50% surcharge and up to 6 weeks delivery time, 6/pkg.
- MF-5164 FEP Teflon Tubing, 0.65 mm OD x 0.12 mm ID, 1 meter (clear)
- MD-1510 Flanged Tubing Connectors, 20/pkg.
- CF-2150 ProClin150™ Preservative, 100 mL
- MD-2250 Intracerebral Guide Cannula and Stylet, 6/pkg.
- MD-1520 Clamp for BR Brain probes
- MD-1521 Clamp Rod (holds MD-1520 and mounts on stereotaxic frame)
- MD-1522 Calibration Station (includes plastic vials and two MD-1520 clamps)
- MD-1300 Acrylic Cement
- MF-5182 Screw Anchors, 100/pkg.
- MF-5362 Drill Bits for Screw Anchors, 5/pkg.
- MF-5176 Trepine Bone Drills, 3/pkg.

Warranty

BR brain probes are warranted to be free from manufacturing defects and viable for a single use. Reuse of probes after insertion into tissue or repeated handling is not guaranteed since this is wholly dependent on the skill of the individual user. BASi is liable only to the extent of replacement of defective items for claims registered within 90 days of the shipping date. BASi will not be liable for any personal injury, property damage, or consequential damages of any kind whatsoever arising from the use of the probe. This warranty does not cover damage to membranes or cannulae through improper preparation, inappropriate connections or faulty handling by the user. The foregoing warranty is in lieu of all other warranties expressed or implied including but not limited to the implied warranties of merchantability and fitness for a particular purpose.

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