MBR Brain Microdialysis Probes

User's Guide

03/07/2002 Update A-1336

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

Introduction

MBR style microdialysis probes and guide cannulae are intended for use in **M**ice, or wherever **M**ultiple and/or **M**iniature implants of **BR**ain microdialysis probes are desired.

A new microdialysis probe must be prepared prior to beginning either an in vitro recovery determination or in vivo sampling study. Preparation of probes involves three basic steps:

- Plumbing and Wetting the Probe: New probes are shipped dry and must be flushed and wetted thoroughly. WARNING! Do NOT soak probes in alcohol. 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 membrane and from all connecting tubing and cannulae.
- Check for Leaks: Examine all connecting tubing, connectors, syringe needles, etc. for any sign of leaking caused by improper or inappropriate connections.

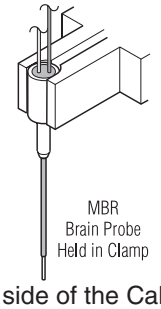
The Dialysis Membrane

The membrane in MBR probes is filled with microscopic pores. During the dialysis experiment, low molecular weight compounds will diffuse through these pores into the probe. A drug may be simultaneously retrodialyzed by the probe, diffusing through the membrane 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. The glycerol must be flushed out prior to a brain microdialysis experiment.

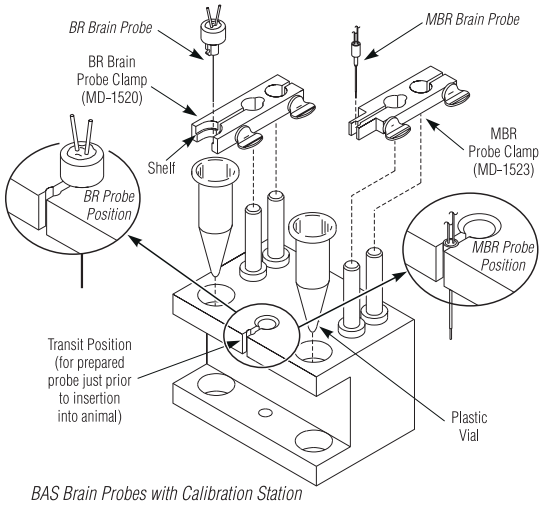
Under no circumstances should the probe membrane be allowed to dry after it has once been wetted. Keep the membrane tip immersed in fluid (water or perfusion fluid) or actively perfused following wetting. The Calibration Station accessory (MD-1524) is useful for short-term storage, probe preparation and in vitro recovery studies. Although MBR probes are not warranted for more than a single use, they may be reused until the membrane is no longer viable or the probe is otherwise damaged.

Probe Preparation Procedure

1. Fill a gas-tight microdialysis syringe (e.g., BAS Bee Stinger, 500 mL) with filtered (0.2 mm regenerated cellulose membrane filter), degassed, room temperature perfusion fluid. Mount the syringe in the pump.
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 care whenever handling a probe. First remove the gray foam cubes holding the probe. Next, carefully invert the opened tray onto your other hand to catch the probe, or reach into the tray to grasp the probe's yellow and green inlet/outlet tubing and pull it straight up. Don't press the probe against the tray since this may damage it.
4. While lightly holding the sleeve protecting the membrane with one hand and the probe inlet and outlet cannulae with the other, gently slip the probe straight out of the protective sleeve.
5. With the Probe Clamp (MD-1523) mounted on the Calibration Station, carefully insert the probe into the U-shaped cavity of the Probe Clamp. Tighten the clamp knob while confirming that the probe is held perpendicularly in the clamp.

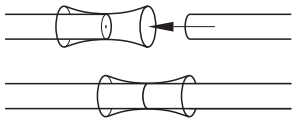


6. Fill a vial on the other side of the Calibration Station with the desired perfusion fluid (artificial CSF, Ringer's solution, etc.) or clean, distilled water. **WARNING! Do NOT soak probes in alcohol.**
7. Using the clamp, transfer the probe to the newly prepared vial of fluid. The probe membrane should now be immersed in fluid. Once the membrane is in solution, it must be kept wet **at all times** to remain viable. If the membrane is not either kept in solution or perfused, the pores in the membrane will dry and the probe will be ruined.



BAS Brain Probes with Calibration Station

8. Flanged tubing connectors (MD-1510) and FEP Teflon® Tubing (MF-5164) should be used for all connections. Cut desired lengths of tubing using a sharp razor blade. Make a clean, square cut. DO NOT USE SCISSORS to cut tubing. Insert tubing halfway into a fresh tubing connector.



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

10. Using tubing connectors, connect the other end of the FEP tubing to the microdialysis probe inlet (yellow) tubing. Avoid any dead space in the connection. If your microdialysis system is so equipped, do not plumb the white, Teflon-lined liquid swivel yet. To maximize flush rates, do not connect any outlet tubing until after step 13.

YELLOW = INLET Cannula
GREEN = OUTLET Cannula

11. Set your pump to flow at 10-15 µL/min and perfuse the probe for 15-20 minutes to flush glycerol out of the pores of the membrane.

12. While the glycerol is being flushed, check for trapped air in the membrane lumen by examining it with a magnifying lens or dissecting microscope. Trapped air is indicated by a meniscus between the liquid and air surfaces. Keep the probe in the probe clamp. Maintain flow through the probe so the membrane remains moistened. If flushing doesn't expel the air, return the probe and clamp to the Calibration Station, then rap the Station on the tabletop to dislodge the air. Repeat as necessary. Do not hit or flick the probe itself.

13. When the probe lumen is bubble-free, return the probe to the Calibration Station and continue to flush per recommendations above to clear residual glycerol from the membrane. Meanwhile, confirm that all connections are leak-free.

14. Now the probe is ready to use. Set the pump to the desired experiment flow rate (usually 0.5 to 5 µL/min). Connect all lines to swivels, fraction collectors, on-line injectors, etc. Start the perfusion again and confirm that there are no leaks at the various connections.

15. For anesthetized animal microdialysis, use the MD-1521 Clamp Rod to hold the clamped probe in your stereotaxic frame.

    For awake animal microdialysis, prior to insertion into an intracerebral guide cannula, first remove the probe from the probe clamp and carefully slip it into the Calibration Station's MBR probe Transit Position. This will facilitate handling the probe while holding the animal. Consult the separate User's Guide included with BAS MBR intracerebral guides for instructions on inserting the probe into the guide cannula.

16. Once the probe has been inserted into the guide cannula, in contrast to the instructions in BAS Awake Animal Containment System manuals, the inlet and outlet FEP tubing should be taped **only** to the upper flag on the tether line. Taping MBR probe tubing to the flag nearest the animal is NOT recommended as this may stress the guide cannula/probe connection.

Probe Storage

New probes should be kept in the original protective packaging until use. When the experiment is complete, mount the probe in the probe clamp and lower it into a water-filled vial on the Calibration Station. Using normal flow rates (1-5 µL/min), thoroughly flush the probe and all connecting tubing and accessories with clean, distilled water to eliminate all residual salts from the system.

Ordering Information

MBR probes are available with 1 and 2, and 2 and 4 mm membrane lengths, with cannulae lengths that match our MBR 5 or 10 mm guide cannulae, respectively. Since both membrane and cannula lengths may differ, in addition to each probe's unique part number, the membrane and cannulae lengths are included in the item's description. For example, a MBR-2-5 probe would have a 2 mm membrane and a cannula matching a 5 mm (MBR-5) guide, while a MBR-4-10 probe would have a 4 mm membrane and a cannula matching a 10 mm (MBR-10) guide. Two mm of a probe's cannula is positioned in the body of the guide cannula, thus the actual cannula length of the probe is 2 mm longer than the 5 or 10 mm of the guide's cannula.

MD-2211	MBR-1-5 Brain Probe, 1 mm membrane. Matches MBR-5 guide. 6/pkg.
MD-2212	MBR-2-5 Brain Probe, 2 mm membrane. Matches MBR-5 guide. 6/pkg.
MD-2232	MBR-2-10 Brain Probe, 1 mm membrane. Matches MBR-10 guide. 6/pkg.
MD-2234	MBR-4-10 Brain Probe, 4 mm membrane. Matches MBR-10 guide. 6/pkg.
CUSTOM	Any other membrane or cannula length
MD-2255	MBR-5 5 mm Intracerebral Guide Cannula and Stylet, 6/pkg.
MD-2256	MBR-10 10 mm Intracerebral Guide Cannula and Stylet, 6/pkg.
MF-5164	FEP Teflon Tubing, 0.65 mm OD x 0.12 mm ID, 1 meter
MD-1510	Flanged Tubing Connectors, 20/pkg.
MD-1523	Clamp for MBR Brain probes and guides
MD-1521	Clamp Rod (attaches to MD-1523 clamp and mounts on stereotaxic frame)
MD-1524	MBR Calibration Station (includes two MD-1523 MBR clamps)
MD-1360	Bone Drills for MBR probes and Guides. For mice or rats. 5/pkg.
MF-5176	Trephine style Bone Drills for MBR probes and Guides. For rats. 3/pkg.
MD-1310	Self-tapping Bone Screw Anchors. For use with mice or rats. 50/pkg.
MF-5362	Drill Bits for Bone Screw Anchors 5/pkg.
MD-1365	Mouse Animal Collars. 100/pkg.
MF-5371	Rat Animal Collars. 100/pkg.
MD-1300	Dental Acrylic Cement
MF-5182	Machine Screw Bone Anchors. For use with rats. 100/pkg.

Pricing and information on BAS microdialysis and liquid chromatography products may be reached from the BAS home page: **www.bioanalytical.com**.

Warranty

MBR brain probes are warranted to be free from manufacturing defects and viable for a single use. Reuse of probes is not guaranteed since this is wholly dependent on the use and handling by the user. BAS is liable only to the extent of replacement of defective items for claims registered within 90 days of the shipping date. BAS 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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