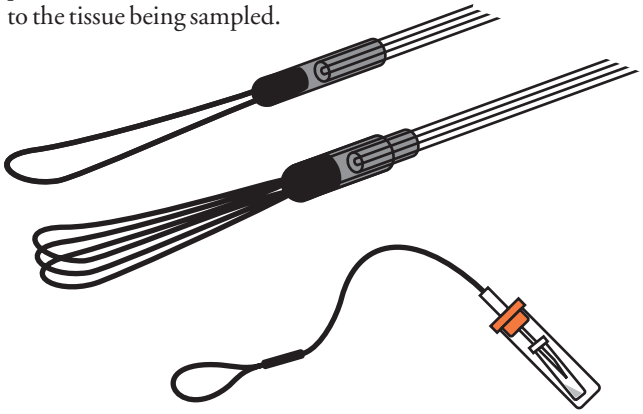
In Vivo Ultrafiltration User's Guide

NOT APPROVED FOR USE IN HUMANS! THESE PRODUCTS ARE DESIGNED SOLELY FOR USE IN EXPERIMENTAL ANIMALS.
Revised 02-06

Both the design and application of in vivo ultrafiltration probes are protected by USA patents 4777953, 4854322 and 5002054, with additional international patents pending.

Introduction

In vivo ultrafiltration (UF) extracts fluid from the extracellular space of living tissue while leaving behind tissue debris, cells and high molecular weight compounds such as large peptides and proteins. It was originally designed to provide continuous tissue sampling in awake, freely-mobile (unrestrained) animals such as dogs and cats. This approach has since been expanded for use in other animals including rats, mice, rabbits, sheep and horses. The targeted tissue is normally subcutaneous tissue, although there is potential application for UF probes in other soft tissues such as the peritoneal cavity, adipose tissue or certain organs. Ultrafiltration sampling is not recommended for use in brain since it depends on the ready availability of fluid which can be replenished rapidly from blood vessels. Disruptions in fluid balance in the living brain can create adverse effects on neurotransmission and normal function. In vivo microdialysis is preferred for brain studies since it neither removes nor adds fluid to the tissue being sampled.



The Ultrafiltration Probe

An ultrafiltration probe consists of one or more loops of hollow dialysis fibers which are joined to a single, non- permeable conducting tube. The tube is joined to a vacuum source which drives the entire ultrafiltration process. The dialysis fibers are placed in tissue and the conducting tube is brought to the body surface. The animal is then allowed to heal and rest for several days. When a vacuum source is connected to the probe, the fluid around the implanted probe is slowly pulled through the dialysis fiber, up the tubing and into a collecting vessel.

In essence, this extracellular fluid is ultrafiltered through the microscopic pores in the fiber. The process permits passage of low molecular weight compounds and prohibits transfer of high molecular weight compounds. The molecular weight cutoff of BASi UF probes is approximately 30,000 daltons.

Sterilization

BASi Ultrafiltration Probes are sold in packages of 6 probes. Probes are individually wrapped in sealed trays and are **not** sterile as shipped.

To sterilize probes using ethylene oxide (ETO), or H_2O_2 (Sterrad), transfer the probes to a gas permeable paper wrapper designed for the sterilization method available. We recommend that the plastic cylinder, which protects

the probe membrane during shipping, stay over the membrane during sterilization.

Recovery

Ultrafiltration frequently yields 100% recovery of a target analyte since the fluid collected is the same fluid that was originally in the tissue. There is no dilution effect, such as that experienced during in vivo microdialysis. However, some molecules may interact with probe materials (membrane, connecting tubing, etc.) causing lower recoveries. An in vitro test should be conducted with new compounds to determine if an interaction exists between the probe materials and target analyte.

Vacuum Source

The vacuum source which drives the ultrafiltration mechanism can be a simple Vacutainer™ used in blood collection. Be aware that the vacuum in such containers may not always be consistent. A certain percentage seem to be defective (i.e. no vacuum). Also be aware that there are multiple types of Vacutainers, some with chemical additives (heparin, EDTA, citrate, etc.) which may be incompatible with the analytical method you have planned. We recommend the types provided with the Ultrafiltration Sampling Startup Kit.

This Startup Kit also includes a large syringe for re-establishing vacuum in a Vacutainer. It is useful if you think you have a defective Vacutainer—pierce the septum with the syringe needle and pull up on the plunger to re-establish vacuum. This would be necessary if you place an autosampler vial inside the Vacutainer for collection of small sample volumes. We don't recommend re-use of Vacutainers.

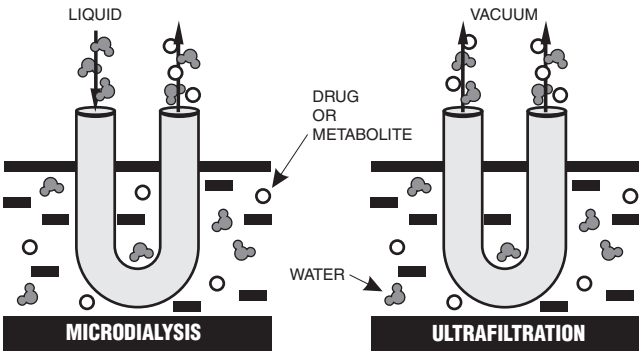
An alternative vacuum source is our MF-5200 mini peristaltic pump. Ultrafiltrates generated by the pump can be routed to a refrigerated fraction collector for uninterrupted sampling. This approach is only viable with a tethered animal and is most useful for rats or mice which are housed in the BASi Raturn or the BASi BeeKeeper Awake Animal System.

Physiological Basis of In Vivo Ultrafiltration

A variety of nutrients, metabolites, toxins and drugs are freely exchanged between the blood and the interstitial space which bathes body tissues. The concentration of any such substance in the interstitial fluid depends on the permeability of the blood capillary membrane to that substance. Lipid soluble chemicals which can dissolve in cell membranes will diffuse rapidly through the blood capillaries. The capillary membrane is also highly permeable to water which diffuses through the membrane and passes through the pores. Water-soluble but lipid insoluble substances must pass through pores in the capillary wall. These 6 to 7 nm pores permit passage of small molecules but prevent passage of most proteins. Capillary permeability will vary from tissue to tissue.

The amount of a particular chemical delivered to a given tissue will depend upon the circulatory dynamics and the perfusion of the tissue by blood vessels. Blood flow to a given tissue is determined by a complex interaction of factors. Autoregulatory mechanisms control blood flow to a tissue to meet the tissue's needs for oxygen, nutrients, and temperature control. Nervous and hormonal control regulate flow to the various systems to meet the needs of the entire animal.

In vivo ultrafiltration removes fluid from the interstitial space at a slow flow rate (generally not exceeding 1 µL/min, e.g. UF-3-12 probe). This fluid is replaced from the blood system. Flow rate depends on the membrane surface area and availability of fluid. In choosing a site for a UF probe, be aware of the factors which regulate flow to that tissue. One of the most convenient places to implant the UF probe is in subcutaneous tissue, but skin has one of the most variable rates of blood flow. This variability is related to body temperature control. New users of the UF technique are frequently surprised at the variability of flow rate from the probe. When the relation between the source of the fluid and its relation to blood flow is reconsidered, this variability is not as surprising. For example, UF in an anesthetized animal will yield a very slow flow rate since all body processes, including blood flow, are abnormally depressed by anesthesia. This also occurs during sleeping or extended rest. When the same animal recovers, eats, drinks and begins to move around, the flow rate will increase dramatically. In many senses, in vivo ultrafiltration is a dynamic method and it provides a truer view of the concentration of analyte in the interstitial space than would be possible from blood sampling alone.



Microdialysis sampling does not affect fluid balance (no fluid added or removed) but does dilute the analyte. Ultrafiltration removes fluid but does not dilute the analyte: the actual extracellular fluid is available for analysis.

In Vitro Studies

UF probes and their accessories are designed primarily for use in living tissue. Initially, you may wish to experiment with the recovery of an unknown analyte in vitro. Before attempting this experiment, you should understand some fundamental characteristics of UF probes.

All dialysis membranes are prepared using a process that creates tiny pores in a plastic tube. These pores emulate the pores in a blood capillary and permit transfer of low molecular weight compounds across the membrane. In microdialysis, the transfer process is diffusion and it is driven by a concentration gradient between fluid inside the membrane tube and fluid outside the tube (the interstitial fluid within the tissue being sampled). In ultrafiltration, the transfer process is driven by a vacuum which forces fluid from the surrounding tissue through the pores and into a conducting tube for collection. The little pores in the membrane are fragile and are kept open by glycerol which lines the interior of the membrane. New UF probes have glycerol in the dialysis fibers to keep the pores open and the fibers pliable.

Most of the glycerol in the probe is removed by the body after the probes are implanted. Glycerol metabolism is a normal and active process in tissue. Most of the probe glycerol will diffuse out of the dialysis fibers and be metabolized by the animal during the time it takes for the surgical sutures to heal (2 or 3 days).

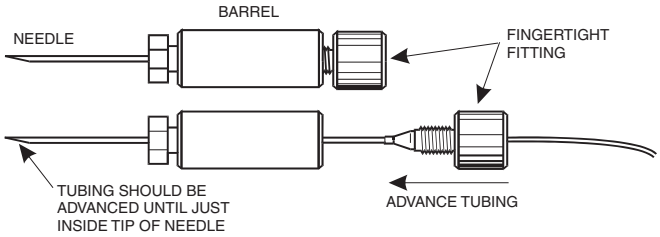
When using probes in vitro, or using them in an animal under anesthesia during the initial implant, you may need to remove the glycerol from the probe by soaking the membranes in distilled water for 24 hours. Glycerol will diffuse out of the fiber pores and be replaced by water.

If a vacuum is inadvertently applied to a new probe in vitro before the glycerol has been removed, the interior of the membrane may look like a chain of little bubbles (string of pearls) and flow may be slow initially. This is because glycerol has been drawn into the joint between the membrane and the conducting tube. Allow time for the viscous glycerol to flow out of the joint and into the tubing. Change Vacutainers if necessary to maintain the vacuum. Flow should establish a more rapid rate eventually.

Hub Assembly

For standard rodent probes, attach the hub assembly by removing the fingertight fitting from the barrel. Slide the probe tubing through the fingertight fitting, barrel and needle. Advance the probe tubing until it is just visible inside the needle tip — don't extend beyond the point. Tighten all connections by hand.

If the white fingertight fitting does not permit passage of the probe tubing, use the clearing rod to push through the fitting (from the wide end towards the narrow end). This should enlarge the opening slightly. Try to insert tubing again. It may also be necessary to disassemble the needle and barrel and then slide the tubing through each part individually. This will require the use of wrenches to loosen the needle from the barrel body.



When assembling the hub with large animal (reinforced) probes, the fingertight fitting is not required. Disassemble the barrel and the needle to expose the ferrule and bushing inside. Slide the barrel loosely over the probe tubing. Slip the probe tubing over the blunt end of the needle (ferrule). Slide barrel back over needle ferrule and tighten the bushing. The tubing is now captured at the needle ferrule.

Holding the hub barrel firmly with one hand and a new Vacutainer with the other, pierce the Vacutainer septum with the hub needle. Slide the needle all the way through the septum. Position the Vacutainer so fluid will drip down into the glass and not across the rubber septum.

If this is a new probe that has been implanted in the animal for several days, you should be able to see the fluid advancing up the conducting tube at a rate of approximately 1 or 2 mm per second.

When a Vacutainer tube has been changed to collect a new sample, flow should appear at the tip of the syringe needle in a few minutes after the vacuum has been re-established.

If There Is No Flow...

1. If this is an in vitro study, was the glycerol removed from probe before vacuum was applied? (see *In Vitro Studies*)
2. Was this probe implanted in the animal long enough for glycerol to diffuse out of the probe?
3. Try a new Vacutainer.
4. If there is still no flow, tighten the hub connections more. Don't overtighten because you can also crimp the tubing and shut off flow completely. You can also try to increase the vacuum by drawing off air in the Vacutainer with the large syringe.
5. If using the peristaltic pump, try increasing the rate. If there is still no flow, change to the hub and Vacutainer system to determine if there are bad connections or leaks somewhere in the system.
6. Is the tubing on the peristaltic pump intact, or is it old, soft and compressed?
7. Are tubing connections viable, or do you see leaks and bubbling at joints?
8. Is the conducting tube on probe crimped, bent or otherwise obstructed? This tubing can not be pulled or stretched. Removing it from a hub assembly may result in stretching and closure of the tubing.
9. It may be time to replace the fingertight fitting or barrel on the hub assembly. These pieces use threaded parts which eventually wear out and stop sealing properly.

Ultrafiltration Sampling Kit

Purchase of the kit is essential before work with UF probes can begin. Kit parts are listed and described below:

MR-5313 **Introducer Needle**, 1/pkg. Use to insert UF probes in subcutaneous tissue of rats, dogs, cats or larger animals.

MF-7021 **Hub Assembly**, 1/pkg. Critical part for use with Vacutainers. The hub is the bridge between the conducting tube on the probe and the vacuum source.

MF-7020 **Replacement Needles** for Hub, 2/pkg. After repeated puncture of Vacutainer septa, the needle will become dull and difficult to insert. Use a replacement needle when this happens.

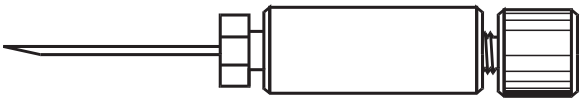
MF-7019 **Clearing Rod**, 1/pkg. Inserted into the hub assembly periodically to open up the fingertight fitting and permit insertion of the probe conducting tube. Insert into fingertight fitting as if it were the probe tubing. Tighten fitting in hub assembly as usual, then loosen and remove rod. The hole in the fingertight fitting should now accommodate the probe tubing more easily.

MF-7024 **Vacutainer Tubes**, 12/pkg. Plain glass, no chemical additives.

MR-2055 **Luer Needle**, 1/pkg. Attached to MR-5022 Syringe

MR-5022 **Luer Syringe**, 1/pkg. Use to create vacuum in dead Vacutainer tube. The Hub Assembly needle would have already pierced the septum on the Vacutainer. Leave it in place while inserting the luer needle from the large luer syringe. Pull up on the syringe plunger to extract air and create the vacuum. Keep pulling on the plunger (this is hard work) while sliding the Vacutainer off the end of the syringe.

MR-5313 Introducer Needle

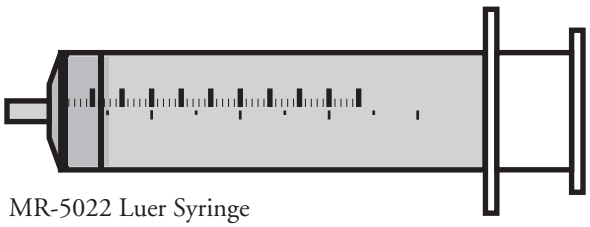


MF-7021 Hub Assembly

MF-7019 Clearing Rod

MF-7020 Replacement Needle

MF-7024 Vacutainer



MR-5022 Luer Syringe

Bubbles in the Ultrafiltrate

After flow is initiated, it is not unusual to see bubbles traveling along the conducting tube with the ultrafiltrate. The source of these is outgassing from the interstitial fluid itself. This is quite normal.

Small Volume Samples

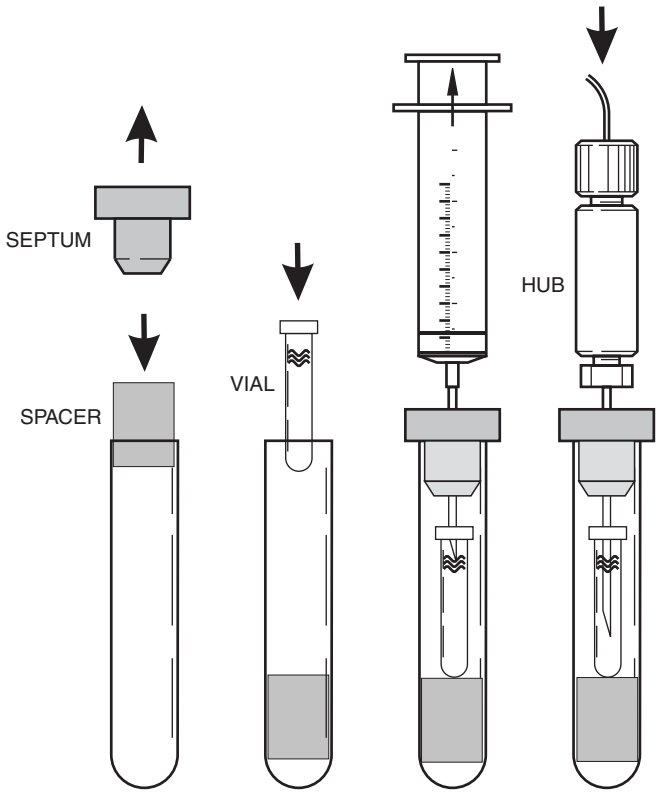
Frequently, ultrafiltration samples are taken over long periods: ranging from one to several hours. This may produce several hundred microliters of fluid.

It is also possible to sample more frequently and collect smaller volume samples. However, it is difficult to aspirate small volume samples from the bottom of a Vacutainer with a syringe. Small glass autosampler vials (e.g. BASi MF-5270) can be placed inside a Vacutainer as follows:

1. Remove rubber septum from Vacutainer.
2. Place a spacer (e.g. cork, cotton) inside vial so that vial will be closer to the septum and the Hub needle will eventually

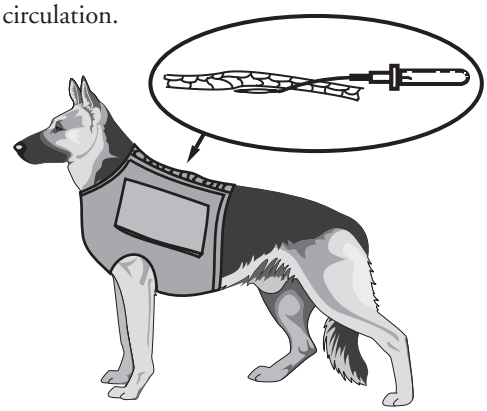
rest inside the vial. Place vial in Vacutainer, with open end towards the open end of Vacutainer.

3. Replace septum.
4. Attach luer needle to large plastic syringe.
5. Insert needle through septum and withdraw air from Vacutainer by pulling on syringe plunger. This is harder than it sounds — brace yourself!
6. While holding the syringe plunger away from the syringe barrel with one hand, use the other hand to slide Vacutainer off the syringe needle.
7. Now insert the prepared hub needle through a different area of the septum and into the open glass vial inside.



Animal Containment

Ultrafiltration can be a completely portable sampling technique. With large animals (dog, cat, rabbit) the hub and Vacutainer can be housed in the pocket of an animal jacket. With a horse or cow, the peristaltic pump can also be used in a jacket since it has battery power as well as AC. Don't let the animal roll on the ground but do let it walk, eat and drink freely to maintain good blood circulation.



Rodents will generally not tolerate jackets or the burden of a Vacutainer. We recommend using a BASi Raturn Awake Animal System, which responds to animal movement with counter-rotation to prevent twists and tangles in lines. The Raturn allows direct connection between the probe and peristaltic pump. If using the BASi BeeKeeper system, a single-channel liquid swivel is sufficient for a single UF probe. BASi FEP Teflon tubing may be connected to the probe, liquid swivel, and peristaltic pump using BASi tubing connectors (MF-5163). In this system, fraction collection can be automated, using a refrigerated fraction collector. The hub and Vacutainer may also be taped to the swivel arm of the BASi Raturn and BeeKeeper Systems.

Microbes Grow in Ultrafiltrates

Ultrafiltrates emerging from the probe are particle-free, protein-free samples which are suitable for direct injection into liquid chromatography systems. However, they are also loaded with nutrients (glucose, amino acids, lactate, vitamins) which make them an excellent growth medium for bacteria. It takes little time for an airborne bacterial spore, multiplying at a logarithmic rate, to proliferate in your sample.

Bacterial growth will produce undesirable waste products which may confound your analytical scheme. If you inject them into your LC system, they may move in permanently. Bacteria may also consume the analyte you wish to measure. This is particularly true for analysis of glucose or amino acids.

Retard bacterial growth by refrigerating or freezing ultra- filtrates as soon as they are collected and until you are ready to analyze them. Frequently clean all parts of the system in contact with ultrafiltrate, including liquid swivels, hub assembly, peristaltic pump tubing, FEP tubing, etc. We recommend an antibacterial wash (e.g. ProClin150™ Preservative), sterilization, and/or frequent replacement.

Subcutaneous Implantation

There are two possible techniques for implanting the UF probe: a one-incision and a two-incision method.

During the **one-incision technique**, the dialysis fiber loop is placed inside the introducer needle. The needle is inserted into the tissue through a single incision and pushed into position. The introducer is then withdrawn backwards over the probe, leaving the loop behind.

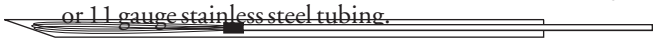
During the **two-incision technique** (illustrated on the next page), the dialysis fiber loop is placed inside the introducer needle. Two incisions are made. The needle enters the proximal incision and exits the distal incision, leaving the probe behind.

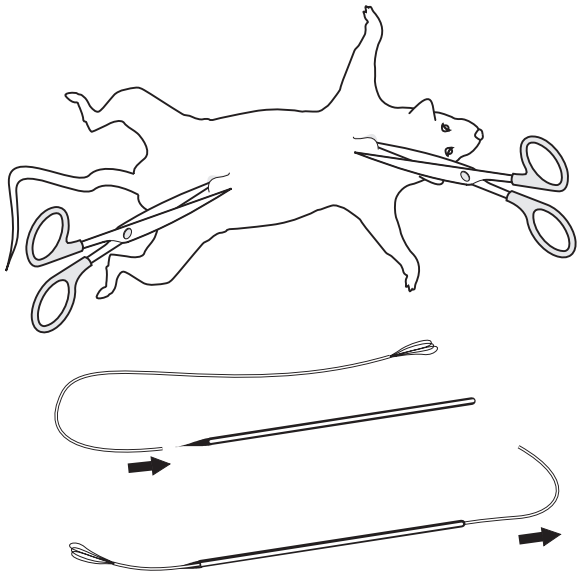
The one-incision method works best for small probes (UF-3-3 or UF-1-2) and small animals. The two-incision approach is preferred for longer UF-3-13 and UF-3-8 probes in subcutaneous locations. It is also necessary to use two incisions if you are using a pre-soaked probe.

Surgical Procedure (Rodents)

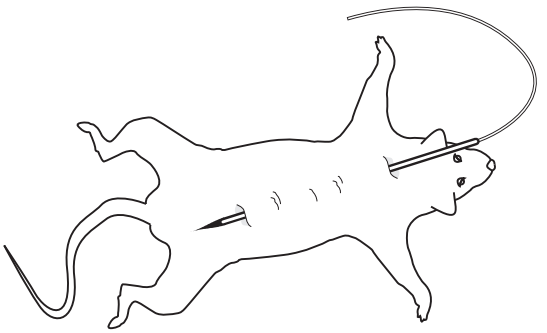
1. Plan to insert the probe at least one day before a vacuum will be applied for sampling. This allows the animal to recover from anesthesia and rehydrate.
2. Anesthetize the animal using a short-acting, injectable anesthetic such as a mixture of Ketamine and Xylazine (1 mL of 100 mg/mL Xylazine into one 10 mL bottle of 100 mg/mL Ketamine). The dose for rats is 0.1 mL/100 g. Inject IP and expect surgical anesthesia within 5 minutes.

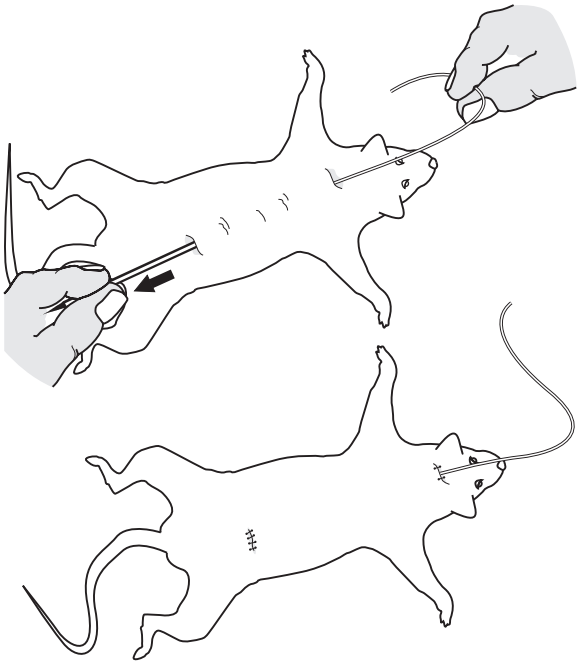
3. Select an insertion site and clip hair from a 2 cm area. Plan to have the probe tubing exit near the base of the neck, behind the animals head. For long term studies, plan to implant the probe at least 1 cm away from the exit incision and tunnel the tubing under the skin to the base of the neck. This will give extra stability to the implant.
4. If a two-incision implant is planned, determine the length of the probe which will be under the skin. Clip a 1 cm circle of fur this distance from the insertion site. Choose a site far enough from the entry point so that the fibers and the tubing joint are under the skin. Make small stab incisions using a no. 11 scalpel blade at the insertion site, the distal site and the tubing exit site on the neck.
5. Place the UF probe inside the introducer needle so that the fibers will end up inside the pointed end. Users of the reinforced probes (MF-7028) will have to construct an introducer, to accommodate these thicker probes, using 10 or 11 gauge stainless steel tubing.





6. Push the introducer needle through the first incision and towards the caudal end. Lift the skin and push the needle under the skin towards the exit site.
7. Hold onto the probe tubing while pulling needle through the exit end. Use lab tape to secure probe tubing to the bench top if you need both hands for this job.
8. If you are using the one-incision method, pull the introducer needle back out over the probe.
9. Re-insert introducer needle into first incision and tunnel towards the neck incision. Thread probe tubing into introducer needle while it is still under the skin. Pull introducer out of the skin, leaving tubing behind.
10. Close all incisions with sutures. Tie one suture around the probe tubing at the neck.
11. Place a plastic collar around the rat's neck and clip off any excess collar material. Attach collar to the wire tether on the BASi Awake Animal System.
12. Connect probe tubing to FEP tubing attached to liquid swivel. Tape tubing to wire guides on the tether. Or, attach probe tubing to hub assembly and use Vacutainer.





Maintenance

For long term sampling with an implanted probe, it is very important to maintain clean connections and a clean implant site to discourage bacterial growth. In large animals, it will generally be more practical to use the Vacutainer approach. Be scrupulous about keeping the hub assembly needle free from contact with any non-sterilized surface. Wipe the septum of a new Vacutainer with alcohol prior to piercing it with the hub needle. In rodents, sampling is often limited to a few days and continuous sampling with automated fraction collection becomes more attractive. Maintenance of clean conditions is then more difficult since the ultrafiltrate must flow through swivels, longer tubing, and a peristaltic pump. After each experiment, disconnect the UF probe and use the pump to flush the system with a ProClin150™ rinse (5 mL ProClin150™ diluted in 1 liter of filtered, distilled, deionized water).

Warranty

BASi warrants in vivo ultrafiltration probes for a one-time use only. Repeated use of the same probe may be accomplished by the careful user, but is not warranted by BASi. In no case will any claim be accepted later than 60 days from the shipping date. In vivo ultrafiltration probes are intended solely for use in experimental animals.

Replacement Parts and Accessories

UF Probes

MF-7023 UF-3-12 Probes, 6/pkg.
MF-7025 UF-3-8 Probes, 6/pkg.
MF-7026 UF-3-2 Probes, 6/pkg.
MF-7027 UF-1-2 Probes, 1/pkg.
MF-7028 UF-3-12 Reinforced Probes for Dogs, 6/pkg.

Accessories

MF-7022 Ultrafiltration Startup Kit
MF-7021 Hub Assembly
MF-7020 Replacement Needle for Hub Assembly
MF-7018 Replacement Fingertight Fitting for Hub Assembly
MF-7019 Clearing Rod
MF-7024 Standard Vacutainers, 12/pkg.
MF-7017 300 µL glass vials, 12/pkg.
MR-5313 Introducer Needle (not suitable for MF-7028 probes)
MR-5022 Luer Syringe

Cleaning Solution

CF-2150 ProClin150™ Preservative, 100 mL

Parts for Automated Fraction Collection

MD-1200 BASi Honeycomb Refrigerated Fraction Collector
MF-5200 Mini-peristaltic pump, battery and AC power
MF-5164 FEP Teflon Tubing, 1 meter
MF-5163 Tubing Connectors, 10/pkg
MF-5271 Polyethylene Plastic Vials, 1000/pkg.
MF-5270 300 µL Glass Vials, 1000/pkg.
MF-5272 Caps and Septa for MF-5270
MF-5274 Vial Crimper

Bioanalytical Systems, Inc.
2701 Kent Avenue
West Lafayette, IN 47906

765-463-4527 765-497-1102 fax
www.bioanalytical.com basi@bioanalytical.com