

# In Vivo Reinforced Ultrafiltration “RUF” Probe MF-7028

## In Vivo Ultrafiltration “UF” Probes

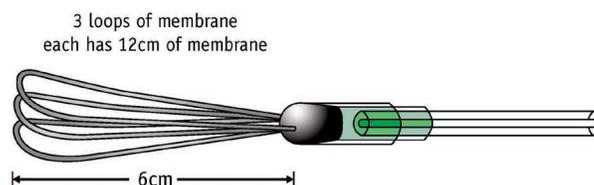
### Introduction

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 moving (unrestrained) animals, such as dogs. 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 other organs. Ultrafiltration sampling is not recommended for use in the 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 (MD) or open flow microperfusion (OFM) are preferred for brain studies since they result in no net change in fluid. Additionally, for studies to examine large molecular weight compounds OFM technique can be applied in place of UF.

An ultrafiltration probe consists of one or more loops of hollow dialysis fibers which are joined to a single, non-permeable collecting tube. The tube is joined to a vacuum source (Vacutainer® or Peristaltic Pump) which drives the ultrafiltration process. The dialysis fibers are implanted in the tissue of interest and the collecting tube is tunneled to the body surface. Following recovery, a vacuum source is connected to the probe, and the fluid around the membrane is slowly pulled through the dialysis membrane, into the collection tube and finally into a collection vial.

### Reinforced Ultrafiltration “UF” Probe

#### RUF 3-12 Reinforced Ultrafiltration Probe



The 12cm membrane in each loop is folded in half, so that the length of the membrane loop is half the length of the membrane, or 6cm in this example.

### Specifications

- Polyacrylonitrile (PAN) membrane, 30KDa MWCO, 320µm OD
- Connector tubing for standard UF probes- FEP, 127cm length, 650um OD
- Connector tubing for reinforced UF probes- PU, 43.7cm length, 1mm OD
- Standard membrane length of 3 loops of 12 cm each
- Custom Probes available upon request

### Features

- Vacuum-assisted, membrane-based sampling
- Can be connected to simple Vacutainer® or to peristaltic pump for collections
- Suitable for long-term (days to months) subcutaneous implantation
- Provides ~100% in vivo recovery
- Directly samples extracellular fluid

### Molecular Weight

Under conditions of equilibrium, the molecular weight cutoff of the membrane on BASi UF probes is 30KDa. To test whether your analyte is an appropriate candidate for using UF probes, it is recommended to perform an in vitro recovery test prior to in-vivo studies.

## Sterilization

Probes are not sterile and cannot be sterilized in their shipping package. UF probes can be sterilized using gas sterilization (ethylene oxide) or plasma sterilization (hydrogen peroxide). To sterilize:

1. Remove the paper seal and transfer the probe tray to a gas-permeable sterilizing bag.
2. UF probes are packaged with a small plastic protective tube over the membrane. Leave this material in place for the sterilization procedure as it will protect the membrane from damage.
3. Once wetted, probes must stay wet to remain viable. Since EtO impacts the membrane lock, this means the probes must be used as soon as possible (preferably within 24 hours) after outgassing to ensure the probe remains viable.

## 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 not dilution effect, such as that experienced during in vivo microdialysis. However, some molecules may interact with the probe materials causing lower recoveries. An in vitro test should be conducted with new compounds to determine if an interaction exists between the probe materials and the analyte of interest (see details below).

## Understanding the 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. Auto-regulatory mechanisms control blood flow to a tissue to meet the tissue's needs for oxygen, nutrients, and temperature control. Nervous and hormonal control regulates 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 $\mu$ L/min). 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 considering the relationship between the source of the fluid and the dependence it has with blood flow, this variability is easily understood. For example, UF in an anesthetized animal will yield a lower flow rate than an awake and moving animal. This is due to the fact that all body processes, including blood flow, are depressed by anesthesia. Lower flow can also be observed during sleeping, surgical recovery, or extended rest periods. As the animal recovers, eats, drinks and becomes more active, the flow rate increases substantially. This reliance on the body's processes means that UF is a dynamic method, and this can provide meaningful information of the analyte concentration of the interstitial space in comparison to blood sampling.

## General Storage Conditions

The probe packaging provides protection from both light and moisture; however, it is not completely impermeable to either. Please store your probes under standard laboratory conditions, avoiding extremes of temperature and humidity.

## Preparing Ultrafiltration Probes

The membrane of an ultrafiltration probe is filled with microscopic pores. During an experiment, the analyte diffuses through these pores into the probe. The pores of new probes are filled with glycerol, which keeps the pores of the membrane moist and open. While in the

membrane, the protective glycerol may affect recovery or interfere with assay results. There are several approaches to removing the glycerol from the probe, as outlined below.

**Option 1.** Preferred Method. When using probes in vitro, for an anesthetized animal study, or for a study where the first samples are needed immediately, the probe can be prepared by soaking overnight in sterile water. Glycerol will diffuse out of the fiber pores and be replaced by water. Make sure that you do not allow the probe to dry out prior to implant.

**Option 2.** When implanting in an animal for an extended recovery period, the probe can be implanted without removing the glycerol. In this scenario, the glycerol exchanges across the membrane at the implant site and will be metabolized by the body during the time that it takes for the animal to heal (2-3 days). Please note that BASi is unaware of any studies to examine tissue inflammation using the method versus the soaking method.

**Option 3.** When a vacuum source has inadvertently been applied to the probe before the glycerol has been removed, the probe can be cleared with continued sampling. In this scenario, the viscous glycerol is pulled into the collection line and must be removed by allowing the flow to continue. Change Vacutainer®s when needed to maintain the vacuum, and observe for an increased flow rate when the glycerol is removed. During this removal period, the membrane and connected tubing may appear to have a chain of little bubbles and slow flow rate.

### Vacuum Source

The vacuum source which drives the ultrafiltration can be as simple as a Vacutainer® attached to the probe tubing outlet. Be aware that there are multiple types of Vacutainer®, including some with chemical additives which may be incompatible with the analytical method in use. Note that unlike blood, no anticoagulant is required for these samples. BASi recommends using a 3mL blank tube to start. This approach is best for large animal studies as it allows the animal to remain untethered and freely moving. The Vacutainer® and

accessories can be mounted in a jacket or vet wrap (as described below), and changed manually as needed.

An alternative vacuum source is a peristaltic pump. Ultrafiltrates generated by the pump can be routed to a [refrigerated fraction collector](#) using a [dual cannula needle](#), for uninterrupted, automated sampling. This approach is only viable with a tethered animal.

### In Vitro Ultrafiltration Studies

When working with new compounds, an in vitro test should be performed to ensure that the analyte is compatible with the UF methodology. It is possible that the membrane will restrict movement of the analyte and it is also possible that the compound will stick to the tubing. Prior to risking animal health and safety, a quick in vitro study can be performed.

1. Prepare the ultrafiltration probe by removing the glycerol locking solution (See [Preparing Ultrafiltration Probes](#), above).
2. Prepare a standard mixture of your analyte. Make sure that the container and volume are able to accommodate the membrane loops for the probe. These loops should be fully submerged in the solution.
3. Connect the probe to a Vacutainer® using the Vacuum Vial Needle Holder ([MD-1322](#)) and begin collecting your sample. If you are using an MF-7028 RUF probe, it will connect directly to the blunt needle on this holder. If you are using any other UF probe, you will need to connect via a tubing connector ([MD-1510 or MD-1516](#)).
4. The volume you collect will be dependent on the amount of time you leave the probe connected. Flow rates of 1-2uL/min should be expected. Collect for a long enough period to gather the volume needed in your analytical method.
5. Remove the Vacutainer® and replace with a second tube. Repeat the same collection period as in Step 4. Taking a second sample will help ensure that you are getting a clean sample without any residual glycerol.
6. Collect a third sample directly from the immersion solution. This “standard” will be used to compare to the collected samples.

If there is a difference between the “standard” and the “samples”, this recovery rate can be applied to your study calculations. Alternatively, a second in vitro study

may be run with shortened collection tubing. If the second study yields better results, it will be clear that analyte is sticking to the tubing. If there is no analyte in the sample, then this suggests that the analyte is unable to pass through the membrane pores.

### **In Vivo RUF Studies: Surgical Procedure for Subcutaneous Probe Placement.**

1. Choose a probe implant site that will minimize the risk from animal interference. When possible, externalize the probe tubing between the scapulae.
2. For large animals, it is recommended to implant two probes- one on each side of the body. This provides a “back-up” probe in case any issues are encountered during the study, while minimizing the number of times the animal is anesthetized and prepped for surgery.
3. Schedule your probe implant surgery at least one day before a vacuum will be applied for sampling. This allows the animal to recover from anesthesia and rehydrate.
4. Anesthetize the animal with your approved anesthetic regimen. Note that this surgery is typically quite fast, so a quick acting or reversible anesthesia is recommended.
5. Clip hair at the incision site. NOTE: It is possible to use either a single incision technique or a two-incision technique for probe implant. If using the two-incision technique, prepare the area for both the insertion site and the trocar removal site, taking care to make the second incision at a distance that will accommodate both the trocar and the loops of the probe.
6. Using scissors or a scalpel, make the incision(s) through the skin to access the subcutaneous space.
7. **Single Incision Technique.** Use the tubing end of the probe (not the membrane) and feed it through the sharp end of the hollow 10Ga trocar until the membrane is seated inside of the trocar. Insert the trocar into the incision, taking care to leave the probe inside of the trocar.
8. **Two-incision Technique.** Insert the 10 Ga trocar into the entry incision and out of the exit incision. To minimize contact with skin and surrounding tissues, place an extra drape near the exit site. Feed the tubing end of the probe into the trocar until the membrane is seated inside the trocar.

9. Remove the trocar from the incision site(s) leaving the probe in place.
10. Suture the probe to the surrounding tissue. It is best to place this suture around the reinforced cuff of the probe.
11. Secure or trim any extra tubing. If trimming the tubing use a new, sharp razor blade or scalpel. This will ensure that the tubing is cut rather than crushed.
12. Close the incisions using your approved skin closure technique.

### **In Vivo RUF Probe Studies: Sample Collection**

The easiest way to collect samples with the RUF Probe is to attach a Vacutainer® directly to the probe. Those instructions are below. If you are working with a tethered animal and would prefer to automate sample collection, please [reach out](#) and we'll help you create a solution that suits your lab.

1. Attach Needle Hub Assembly directly to the probe using a press-fit of the tubing onto the blunt end of the Hub. Press the tubing until is in contact with at least 3-4mm of the Hub.
2. Swab the cap of a blank 3mL vacuum vial with alcohol and then apply to the Needle Hub Assembly by pressing it onto the sharp needle.
3. Fluid will not flow immediately- there is a delay between application of the vacuum and sample collection as the interstitial fluid travels through the probe.
4. At completion of sampling period, remove the vacuum vial and replace for the next collection period.
5. Always keep a vacuum vial connected to the probe, even if the sample will not be needed for the study. This ensures that the probe maintains flow and is not exposed to pathogens.
6. There are several techniques that can be used to secure the vacuum vial to the animal:
  - a. **VetWrap.** Use sticky veterinary wrap around the neck or trunk of the body to hold the vial in place. This is low cost, but risks being rubbed/chewed off.
  - b. **Adhesive Pocket.** An adhesive pocket made of non-absorbent material can be attached directly to the skin with the vial inserted. This is low cost, but risks being rubbed/chewed off, and may cause irritation depending on the adhesive.

- c. **Jacket and Pocket.** An animal jacket, such as those provided by Lomir, can be placed on the animal. Jackets can be customized with a fabric pocket and openings to feed the tubing from skin to pocket. This is a higher cost, but the best option to protect animal and probe. Jackets are reusable.

## Troubleshooting

**Bubbles in the line.** This is typically a sign that the glycerol lock was not properly removed before use, but can also occur following EtO sterilization of the probe. The solution is to apply a vacuum until the glycerol has been removed from the probe. Attach a vacuum vial, and replace as needed to maintain the vacuum. Observe for an increased flow rate as well as removal of the chain of bubbles when the glycerol is removed.

**Slow or reduced flow.** This can be a sign of a few mechanical issues. Start by checking that the tubing isn't kinked. If there are no visible issues with the tubing, then change the Vacutainer® to ensure that the issue is not related to a loss of vacuum. An obstructed needle can also cause slow flow, which can be resolved by replacing the Vacuum Vial Needle (MD-1320). However, this issue can also be related to the animal/tissue. Ultrafiltration flow rate depends on the membrane surface area and availability of fluid at the site. If your animal is moving less, is dehydrated or has been treated with a compound that impacts normal physiological function, you may see a resulting change in the amount of fluid being collected.

## Warranty

Ultrafiltration ("UF") Probes are NOT for use in humans. These products are designed solely for preclinical research and are viable for single use. BASi warrants its products against manufacturer defects. BASi is liable only to the extent of replacement of defective items for claims registered within 90 days of the shipping date. For additional details about our warranty and terms of sale, please see [here](#).

## Ordering Information

### Reinforced Ultrafiltration "UF" Probes

MF-7028      UF-3-12 Reinforced Probes for Large Animal Implants

### Accessories

MD-1322      Vacuum Vial Needle Holder  
MD-1320      Replacement Vacuum Vial Needles, 6/pk  
MD-7024      Untreated 3mL Vacutainer®s  
MR-5313      Tissue Introducer Needle

Vacutainer® is a trademark of Becton-Dickinson Corp, Rutherford, NJ.

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