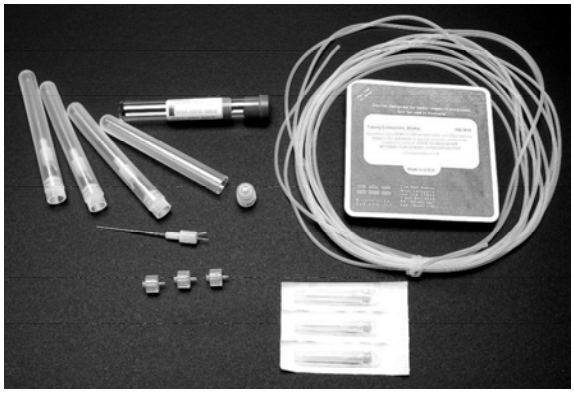
MF-7040 User's Guide
Vacuum Needle Kit for Ultrafiltration Sampling



Kit Contents

Qty.	*Part No.*	*Description*
4	MF-7016	Disposable Ultrafiltration Vacuum Needle
1	MD-1510	Flanged Tubing Connectors, pkg of 20
5 meters	MR-5087	Silicon Tubing for Connection to Trap and Vacuum
1	MF-7033	Plain Vacutainer, 10 mL
3	MR-2068	Luer Needle
3	MR-4424	Luer Connector

Other Items Used with this Kit

Qty.	*Part No.*	*Description*
1	MF-7023	BASi Model UF-3-12 Ultrafiltration Probes, pkg of 6
1	MF-7025	BASi Model UF-3-8 Ultrafiltration Probes, pkg of 6
1	MF-7026	BASi Model UF-3-2 Ultrafiltration Probes, pkg of 6
1	MF-7027	BASi Model UF-1-2 Ultrafiltration Probes, pkg of 6
1	MF-7028	BASi Model RUF-3-12 Ultrafiltration Probes, pkg of 6
1		Laboratory Vacuum Source such as a Vacuum Pump
1	MD-1201	BASi HoneyComb: Dual-Needle, Refrigerated Fraction Collector
1	MF-5270	Borosolicate Glass Sample Vials, 300 µL, 6 x 32 mm, pkg of 1000
1	MF-5273	Plastic Snap-Cap for MF-5270 Vials, pkg. of 1000
1		BASi Raturn Interactive Caging System
10 mL		70% Ethanol (Ethyl Alcohol) (CH_3CH_2OH)

Introduction

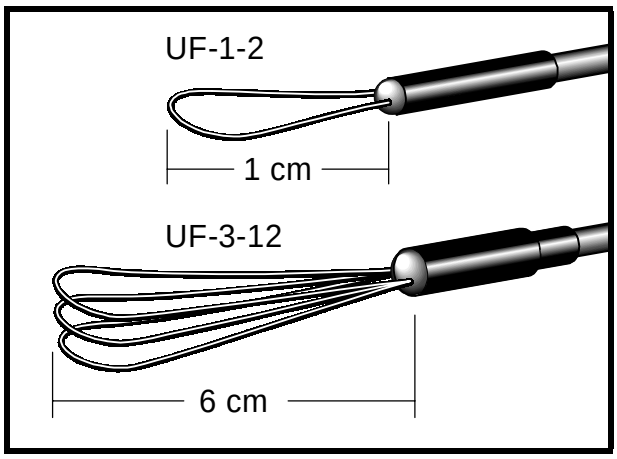
This kit provides the means to collect ultrafiltrates into standard glass vials on a HoneyComb refrigerated fraction collector. The advantages to this method include:

1) automation of *in vivo* ultrafiltration sample collection
2) the ability to collect small volume samples with precision
3) simultaneous collection from two separate ultrafiltration probes
4) refrigeration of the collected ultrafiltrates
5) better control of applied vacuum

In vivo ultrafiltration relies on use of an ultrafiltration probe and a vacuum source to withdraw extracellular fluid from living tissue in a freely-moving subject. The fluid can't be collected at a rate exceeding the rate of its replenishment by blood vessels within the same tissue. Therefore, collection of ultrafiltrates provides more than just a fluid for analysis. It also provides an indirect indication of blood flow to a particular tissue. Timed collections of weighed ultrafiltrates can provide a useful index of blood flow to a particular tissue. This kit provides a needle designed for use with the BASi HoneyComb fraction collector. When used as described, this instrument can automatically collect ultrafiltrates into as many as 999 sealed and refrigerated vials at a preset interval ranging from 0.1 minutes to 99 hours.

About Ultrafiltration Probes

BASi ultrafiltration probes have model numbers which describe their construction. Take for example the models UF-1-2 and RUF-3-12. The designation UF means a standard ultrafiltration probe suitable for use with rodents, while the term RUF means a reinforced ultrafiltration probe for use in dogs, sheep and other large mammals. The numbers tell you the number of membrane loops used in the probe and the length of the membrane in each loop. For example, UF-1-2 is a standard probe with one loop of membrane that is 2 cm in total length. Because the membrane is folded into a loop, the length of the loop itself will be half the length of the membrane, in this case 1 cm. The RUF-3-12 probe is a reinforced probe with 3 loops of membrane each being 12 cm long. The loop will be 6 cm long. Ultrafiltration flow rates increase with the surface area of membrane, so the UF-1-2 probe will produce much slower flow rates than the RUF-3-12 probe.



Principles of Operation

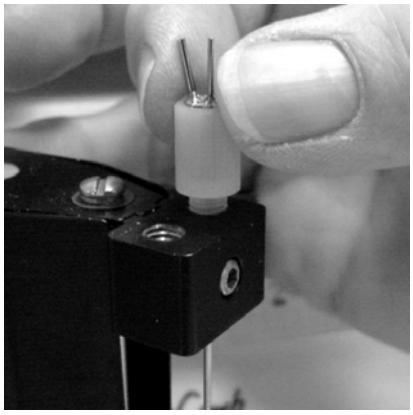
Ultrafiltration probes have typically been used with vacutainers which provide the vacuum source needed to withdraw fluid from tissue. The vacuum needle accessory creates a mini-vacutainer every time it pierces the septum of a sealed collection vial by evacuating all of the air inside the vial. The central cannula on the needle is attached to the ultrafiltration probe, while the side cannula on the needle is attached to a vacuum source. To protect the vacuum source from contamination, a simple trap is constructed of a vacutainer, luer needles, luer connectors and tubing. Two vacuum needles may be connected to one trap and one vacuum source.

The vacuum needle is intended to be a disposable item. Use of a new needle is recommended to avoid potential problems with drug carryover during pharmacokinetic studies, or bacterial growth inside the needle between studies. Once in use, a vacuum should continue to be supplied to the needle in order to promote continuous flow through the needle. This is particularly important to avoid bacterial contamination which can influence concentrations of biomarkers (e.g. glucose, amino acids, other nutrients) in the collected samples.

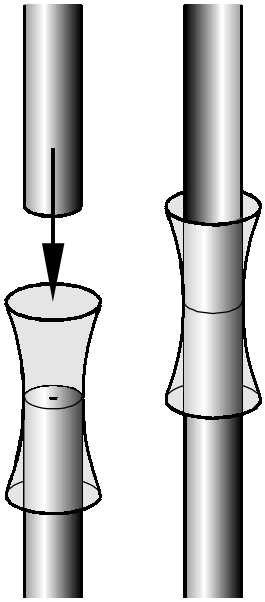
Installing The Needle

1. Locate the needle carrier on the BASi HoneyComb fraction collector. One of the two needle positions may be blocked with a set screw if you are using a collector from the Culex Automated Blood Sampler. Remove set screw with an Allen wrench.

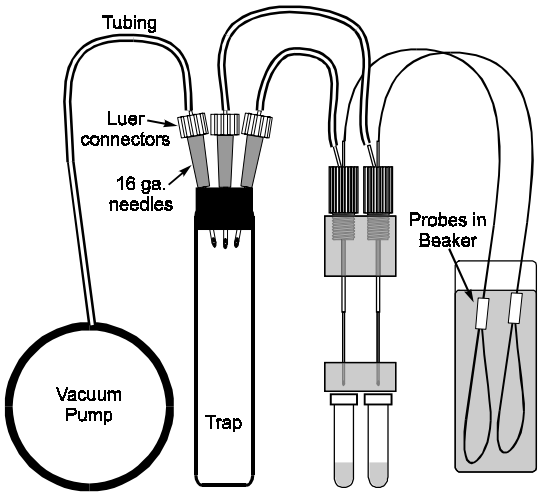
2. Insert one or both vacuum needles into the needle carrier by hand. Do not use tools. Advance the needle until it just touches the top of the needle carrier. Do not overtighten.



3. Soak the MD-1510 tubing connectors in 70% ethanol for at least 30 minutes. Remove one connector and attach one end to the conducting tube of the ultrafiltration probe and the other end to the center cannula on the vacuum needle as shown. Allow the alcohol to evaporate completely to ensure that the connector has fully contracted to form a tight seal between the probe and the needle.



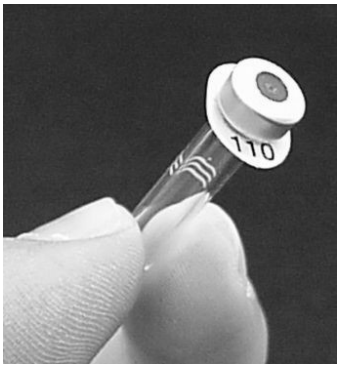
4. Cut a convenient length of tubing to make the connection between the side cannula on the vacuum needle and the trap. Insert the tubing over the vacuum needle and place one of the luer connectors into the other end of the tubing. Attach one of the needles to the tube and use this needle to pierce the septum of the vacutainer. The vacutainer will serve as a trap to contain spills from an overfilled vial and protect your vacuum source.



5. Prepare another piece of tubing with a connector and needle and pierce the septum of the vacutainer again. The other end of the tubing must be connected to the vacuum source in the lab. Since the nature of the vacuum source is unknown to us, we rely on your ingenuity to make this connection. We have used combinations of luer connectors and pipette tips to fashion a nozzle that will fit over a vacuum spout.

6. If you wish to connect the second vacuum needle, repeat steps 1 to 4.

7. Load capped vials into the fraction collector. If you wish to weigh the ultrafiltrate, we recommend the use of Chads for Vials as a way to label each vial and provide a convenient handle for transfer in and out of the balance.



Chad labeled vial.

Operation

1. Vacuum levels above 15 in Hg tend to produce outgassing (bubbles in the ultrafiltrate), so we recommend a level less than this if you have a way to monitor or adjust your vacuum pump.

2. Raise the fraction collector needle and place one sealed vial in the “W” positon on the fraction collector. Start the vacuum pump and then lower the needle using the needle Up/Down button on the front panel of the HoneyComb.

3. Collect a sample for approximately 5 to 15 minutes and then raise the needle using the needle Up/Down button. Remove and inspect the vial to make sure you have established flow from the probe.

4. Set the fraction collector to the number of samples and the sampling interval desired. If you exceed 48 vials, you will have to retrieve the filled vials periodically and replace them with empty vials while the fraction collector is still operating.

5. Caution! Do not attempt to remove a vial in close proximity to the needle while the instrument is running. Keep an eye on the countdown clock on the fraction collector and don’t remove/replace vials as time is running out on a collection.

6. If you are using both fraction collector needles, change the fraction collector to dual cannula (2 needle) operation so the carousel will advance two vials at a time instead of one.

7. Press the Run/Stop button to initiate timed collection of ultrafiltrates as programmed.

8. If you see fluid accumulating in the trap, your interval may be too long for the flow rate at this level of vacuum. Adjust the interval or the vacuum as needed.

Studies With Awake Animals

This method of collection assumes the animal is caged in a Raturn Interactive Cage. This cage allows the ultrafiltration probe tubing to remain intact from the animal to the fraction collector, until no intervening device that could break the vacuum. Vacuum unltafiltration will not work through liquid swivels, which can’t maintain the vacuum reliably.

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