

Procedure for cleaning U-Probes

Enzymatic Detergents:

After the U-Probe has been implanted and removed, it is critical that any proteins, lipids, blood, or other biological matter be removed in a timely manner. If the U-Probe is not immediately cleaned, these substances may dry on the end of the probe, covering the sites. This dried layer will serve as an insulator and greatly increase the impedance in future experiments, resulting in decreased signal quality.

Plexon recommends using either *Metrex EmPower Dual-Enzymatic Detergent* (available at www.metrex.com) or *Tergazyme* from Alconox to clean U-Probes after each use. These detergents contains protease enzymes which remove a variety of proteins, and surfactants to remove carbohydrates, lipids, and other proteins. When used properly, the detergent will not damage the electrode sites. See the manufacturer's webpage for more information.

****Be sure to follow all safety precautions on the container****

1. Dilute the detergent with warm water in the appropriate ratio (1:128 for Metrex) and swirl to mix. The enzyme activity is affected by temperature, so only water between 68°F and 104°F (20°C - 40°C) should be used. A fresh detergent solution should be made for cleaning after each experiment.
2. Stir the U-Probe in the diluted detergent for approximately 30 seconds and then allow it to soak for 30 minutes.
3. Remove the U-Probe and stir it in distilled water to remove any detergent residue that might remain.
4. Allow to air dry and replace the U-Probe in its container.

If it is not possible to immediately clean the UProbes in the recommended enzymatic detergents after use, the U-Probe should be submerged in water until it can be thoroughly cleaned. Please contact Plexon for any questions regarding the cleaning of the U-Probe.

Physical cleaning with an alcohol pad:

In addition to the Enzymatic Detergents, the U-Probe can be cleaned with isopropyl alcohol pad. Use an isopropyl alcohol wipe/prep pad (medium size: 1 1/8 inch X 2 3/8 inch). Open the envelope; fold the pad into two at the middle. Grab it between the thumb and index finger, so that the folding edge looks towards your palm and it opens upwards. Place the U-Probe between the fingers

with the wipe, close fingers gently on the U-Probe with the wipe, and pull the U-Probe out of the fingers straight, gently and slowly. Repeat it several times, rotating the U-Probe so that all sides are cleaned.

Procedure for Sterilizing U-Probes

1. Put the U-Probe shaft and tip into diluted Nolvasan disinfectant solution for 1 hour
2. Run purified, distilled water over the Uprobe

Instructions to load the fluid channels on the U-Probe:

Materials:

- 25 - 26 gauge hypodermic needle to fill up the inlet tube.
- 0.28mm inner diameter inlet tube (PORTEX Polythene).
- 5microL Hamilton syringe for drug delivery (Type 75, N. tip 2).
- Dispenser that fits the Hamilton, home made, but you can buy the Hamilton DS Digital Dispenser.
- 1mL syringe for filling.
- 5-10mL syringe for cleaning.

First make 3 sets of inlet tubes, one for the drug delivery, one for air cleaning and one for fluid cleaning. Attach the 0.28mm tubes (10-30cm long) to the 25 or 26gauge needles, so you get 3 sets, be sure you don't puncture the tube and be sure it doesn't leak at the junction.

The fluid going into the probe has to be distilled water or isopropyl alcohol based solution with no solid part (i.e. water-soluble or isopropyl-soluble). Use a 0.2micron syringe filter to get rid of the sediments before putting it into the probe.

Fill up the tube with the drug solution. Use 1mL syringe. First fill up the 1mL syringe, and be sure no air inside. Take the 25-26gauge needle and guide some drops into the inlet of the needle from the 1mL syringe. Aim it to the side of the inlet, it helps you in preventing air bubbles going in. If the inlet is filled, attach the 1mL syringe so, that no air goes in. Gently push the syringe, so you can see that the fluid goes slowly into the tube. Use plenty of light to see the front end of the fluid. If you see air, let in enough fluid that pushes out the bubble. If no bubble in the tube take it down and start to fill up the Hamilton.

Filling up the Hamilton so that it doesn't contain air is also tricky. It is a good idea to practice before the actual experiment. You should draw up the solution slowly, then suddenly push back the knob of the syringe with your index finger. Push it fast, but not too long. You will see air going out. Repeat pulling and pushing on the knob until no air comes out. Keep the needle of the Hamilton in the solution all the time during filling.

After you're done with filling up the Hamilton, put it into the dispenser. Let out some fluid, so you can see a drop at the end of the needle. Take the filled tube/needle/1mL syringe complex and attach it to the inlet of the electrode. Gently push the tube on the inlet needle, be sure it's not leaking. Now you can try to inject the drug solution across the system. Use a microscope to see if the solution coming out at the other side. Push out enough fluid to get rid of bubbling.

Now comes the tricky part - you have to attach the Hamilton to the tube. To do that, first you have to remove the needle from the tube. Push gently on the 1mL syringe and gently remove the tube from the needle. If you are lucky it comes

down without air going in. In this case pick up the Hamilton with the dispenser, push out a drop of fluid and attach the free end of the tube to the Hamilton. Practice makes it easier. Now you have the working setup.

Inject some fluid to see if it works. Before implanting, dip the U-Probe with the fluid channel into distilled water and swab it with a wet cotton tip applicator (ear cleaner) to get rid of the contamination.

A typical injection volume is 0.5-1microL. If the U-Probe is in the brain, you can see from the recordings that the injection was successful. After good injection, there will be plenty of saturation or DC shift around the injection site.

Procedure for cleaning the fluid channels on the U-Probe:

After you're done with the recordings and injections with the U-Probe, gently pull it out of the brain and dispense the remaining fluid from the Hamilton. Fill up the second tube/needle and 5-10mL syringe with distilled and filtered water, remove the tube from the electrode and attach the cleaning tube to it. Push plenty of cleaning water out. Be sure that the water comes out. Remove the tube from the electrode and now push air from the third tube/needle line, until only air comes.

Some figures:







