

DEEP WELL LIBRARY REPLICATION

Disinfecting:

The Deep Well Replicators can be sterilized by hot air oven, autoclaving, treating in 10% bleach or alcohol flaming. The pins can be cleaned between source plates by dipping briefly in a 10% bleach solution, followed by a series of two sterile dH₂O baths (all in tip lid boxes), then a 99% isopropanol bath in a nonflammable VP 420 glass reservoir. The pins can be air dried, using a hair drier or by flaming the isopropanol. Between baths, remove the liquid from the pin tips by blotting on a lint-free blotting paper (VP 522) as lint from paper towels will interfere with uniform liquid transfers. This blotting step is also very important to reduce carry over.

If you flame, do not blot the isopropanol before igniting and be prepared for a "whoosh" upon ignition. Keep the isopropanol reservoir and blotting paper at least 3 feet from the flame. We strongly advise you to use a non-flammable alcohol reservoir such as the VP 420 Pyrex® alcohol reservoir to avoid laboratory fires. If you don't flame, blot on the lint-free blotting paper and let the isopropanol evaporate. The evaporation can be speeded up with a hair dryer. It is important that the pins be dry before going into the next source plate.

**** Flaming just entails igniting the alcohol on the pins and NOT heating the pins directly in a Bunsen burner which can damage the corrosion resistant properties of the stainless-steel pins.**

Care:

Before each day's use we recommend that the pins be cleaned with VP 110 Pin Cleaning Solution, which is designed to clean stainless steel and condition the pins. If the pins should be coated with organic material, they can be mechanically cleaned with the VP 425A brush and Ivory dish detergent. If you have access to an ultrasonic bath, we recommend using MICRO 90®, from Cole Parmer®, at a 1/100 dilution in the ultrasonic bath. If you use an ultrasonic bath, hold the Replicator in the bath without letting the pins touch the bottom of the reservoir (the vibrating bottom surface of the sonicator's reservoir may damage the pin tips). The Cole Parmer Catalog #P-08857-02 Ultrasonic Cleaner is ideal for cleaning the Replicators.

It is not necessary to clean the replicators in an ultrasonic cleaner if you clean the pins with bleach and brush with detergent after each day's use.

Use: (liquid to liquid and liquid to membrane transfers)

1. Place a deep well LIBRARY COPIER™ (VP 381A) over a 96 deep well source plate with the single alignment hole side of the device closest to row H of the plate. Slide the deep well LIBRARY COPIER™ to make sure the plate is seated within the device and therefore registered.
2. Place a second VP 381A deep well LIBRARY COPIER™ over a 96 deep well reception plate with the single alignment hole side of the device closest to row H of the plate. Slide the deep well LIBRARY COPIER™ to make sure the plate is centered and seated within the device and therefore registered.
3. Hold a sterile 96 pin deep well MULTI-BLOT™ Replicator (VP 408A, VP 408FA, VP 408B or VP 403F) at a 45° angle to the source plate deep well LIBRARY COPIER™ and 20° angle to the left alignment hole. Place the right guide pin into the right alignment hole. Slowly decrease the 20° angle and place the left guide pin into the left alignment hole. Rotate the Replicator forward until guide pins line up vertically and slide down the alignment holes and the Replicator pins drop into the wells (see diagram).
4. Hold the LIBRARY COPIER™ in one hand and mix contents of wells by raising and lowering the Replicator 3X through the meniscus with the other hand. The speed at which the pins are removed from the wells on the final withdrawal will affect the size of the hanging drops and the amount of liquid on the sides of the pin. Removing the pins quickly from the source plate produces large, hanging drops on the tips of the pins and more liquid on the sides. We recommend removing the pins on the final withdrawal at a slow even speed each time (~.5 cm/sec). This action produces very uniform transfers from plate to plate and reduces the amount of liquid hanging on the tip and sides of the pins. Performing this operation with the LIBRARY COPIER keeps the pins in the middle of the well and prevents hanging drops from being accidentally touched off.
5. To deliver to another microplate with liquid in the wells, dip and raise the pins 3 times through the recipient plate's meniscus. Blot the pins on lint-free paper to reduce carry over if the pin tool is put back into the source plate or to the wash bath.
6. Repeat steps 3-5 for each replicate plate as needed.
7. To deliver to a standard 96 well plate or to a 384 well plate place a VP 381B Standard microplate LIBRARY COPIER over the reception plate and repeat step 5 into the reception plate. The VP 381B LIBRARY COPIERs are designed to index to the deep well Multi-Blot Replicators with their larger guide pins to standard 96 and 384 microplates. Using the proper alignment holes you can transfer to all 384 wells with a 96 pin tool.

8. To deliver to a membrane, have a soft absorbing pad under the membrane (VP 522, VP 521, or VP 521V) and press gently on the replicator. We recommend using one of our VP 381B LIBRARY COPIER registration systems to prevent the pins from making skid marks on the membrane.
9. Repeat steps 3, 4 and 8 for each replicate blot as needed.

Test your Replicator using dye (5% red food coloring) in 10 mM Tris, pH 8.0 with 0.005% Sarcosyl or Tween 20 as wetting agents in water.

Note: If you are having problems with varying volumes of liquid on the tips, clean the pins with the [VP 110 Pin Cleaning Solution](#). Cleaning with the V&P Pin Cleaning Solution will reduce the surface tension on the pin and this will solve 99% of your pin loading problems. Also, you can add 0.005% Sarcosyl, Tween 20, protein or carrier DNA to lower the liquid surface tension.

Warning:

- **Do not soak in bleach solutions for a long period of time as this can corrode the stainless steel pins.**
- **Do not soak in deionized water as this can corrode the stainless steel pins.**
- **Do not heat the pins directly in the Bunsen Burner flame**

The VP 110 pin cleaning solution contains a dilute acid solution which can strip the protective anodized surface off the anodized aluminum replicator bases or float plates. If you accidentally get the VP 110 pin cleaning solution on an anodized surface, quickly rinse it off with water.