

ROBOT PIN TOOL CLEANING AND LIQUID SAMPLE TRANSFER

OVERVIEW

There are several key steps in the successful use of pin tools:

1. The first and most important step is to start with clean pins. It is strongly recommended that V&P's specially developed VP 110A Pin Cleaning Solution is used at the beginning and end of each day to keep the pins clean.
2. Another key step is blotting onto a lint-free blotting material after delivery to the last daughter (recipient) plate and between wash solutions. Blotting greatly reduces carry-over. Blotting with lint-free blotting paper ensures that the pins do not pick up lint which will affect the volume transferred. V&P carries several different formats of Lint-Free Blotting Media.
3. Three wash solutions are recommended. The wash solution in the first reservoir will depend on the type of sample being transferred:
 - a. For nucleic acid and biological (cell) transfers, use 10% bleach.
 - b. For peptide and protein transfers, use 0.1% SDS.
 - c. For small molecule compound transfers, use 50:50 DMSO: distilled H₂O or other solvent. The second solution can be distilled H₂O, or 100% alcohol if transferring small molecule compound libraries. Alcohol is recommended for the last wash solution due to its solvent characteristics and fast evaporation rate. NOTE: Always use distilled H₂O, not deionized H₂O in all pin tool applications. Long term exposure to deionized H₂O will damage the stainless-steel pins. V&P offers a variety of wash reservoirs: static, flowing, disposable and fountain reservoirs. Combination wash and blot reservoirs are also available.
4. When dipping the pins into mother (source) or daughter (recipient) plates and wash solutions, it is important to triple-dip into each solution. This mixes the liquid, loads and washes the pins to give more reproducible results. The pins should be completely removed from the liquid prior to each dipping to achieve maximum effect.
5. The speed of pin withdrawal from the liquid during the final dip will determine the volume transferred. The speed of the pins during the first dipping (washing/mixing) steps can be fast (2-8 cm/sec).

PROTOCOLS

Cleaning and Liquid Sample Transfer with V&P Scientific Robot Floating Pin Tools

THREE CLEANING/TRANSFER PROTOCOLS:

- I. Routine or Daily Cleaning (page 2).
 - i. Before starting an experiment and after finishing an experiment.
- II. Liquid Sample Transfer and Cleaning Between Transfers (page 3).
 - i. Example of liquid sample transfer protocol from one mother plate to one daughter plate.
 - ii. Recommended cleaning after sample transfer for prevention of sample cross-contamination during an experiment.
- III. Heavy Duty Cleaning for Neglected Pin Tools (page 4).
 - i. When the pins have a buildup of material after extensive use without cleaning.

Appendix 1. V&P Scientific Pin Tool Cleaning Accessories (page 6).

I. ROUTINE OR DAILY CLEANING:

Before starting an experiment and after finishing an experiment.

- A. Set up three wash reservoirs:** Use VP 540-1 Wash Reservoirs or other appropriate reservoirs.
1. Fill wash reservoir #1 with 1:5 dilution of VP 110 Pin Cleaning Solution (please see Technote 40 for more information about VP 110).
 2. Fill wash reservoir #2 with distilled H₂O.
 3. Fill wash reservoir #3 with alcohol (ethanol, isopropanol, or methanol).
 4. Adjust the level of wash solution in each reservoir as follows (example volumes are for VP 540-1):
 - a. The first reservoir should wash slightly higher on the pins than the “high water mark” which results from dipping into microplates during an experiment (195 ml).
 - b. The second wash reservoir should be slightly higher than the first reservoir (200 ml).
 - c. The third wash reservoir should be slightly higher than the second reservoir (205 ml).
 5. Next to each wash reservoir place a VP 540DB Heavy Duty Blotting Station.
- B. Washing pins:** It is critical for the tip of the pin to move up and down through the meniscus with each dip into the wash solutions.
1. Dip pins into a 1:5 dilution of VP 110 Pin Cleaning Solution 3 times through the meniscus. Blot pins on VP 540DB Blotting Station.
 2. Repeat wash and blot with VP 110 Pin Cleaning Solution.
 3. Dip into distilled H₂O wash, 3 times through the meniscus. Blot pins on VP 540DB Blotting Station.
 4. Repeat wash with distilled H₂O.
 5. Dip into alcohol wash, 3 times through the meniscus. Blot pins on VP 540DB Blotting Station.
 6. Repeat wash with alcohol.

C. Drying pins: If pin tool is cleaned while installed on a workstation, the pins can be air dried for a few minutes or dried over a fan or vacuum station for one minute. See this link for the VP 904 Pin Dryer: http://www.vp-scientific.com/Pin_Tool_Robot.php#VP904. Solid pins do not need to be dried with a fan or vacuum if they are blotted as the alcohol will evaporate very quickly. Slot pins will require a little longer time to dry without a fan or air movement.

II. LIQUID SAMPLE TRANSFER AND CLEANING BETWEEN TRANSFERS:

A. Set up three wash reservoirs in VP 540-1 Wash Reservoirs or other appropriate reservoirs:

1. Wash solution in reservoir #1 will depend on the sample being transferred:
 - a. For nucleic acid and biological (cell) transfers, use 10% bleach.
 - b. For peptide and protein transfers, use 0.1% SDS.
 - c. For small molecule and compound transfers, use 50:50 DMSO:distilled H₂O.
2. Fill wash reservoir #2 with distilled H₂O (or 100% alcohol, if transferring compound libraries.).
3. Fill wash reservoir #3 with alcohol (ethanol, isopropanol or methanol).
4. Adjust the level of wash solution in each reservoir as follows (example volumes are for VP 540-1):
 - a. The first reservoir should wash slightly higher on the pins than the "high water mark" which results from dipping into microplates during the experiment (195 ml).
 - b. The second wash reservoir should be slightly higher than the first reservoir (200 ml).
 - c. The third wash reservoir should be slightly higher than the second reservoir (205 ml).
NOTE: The VP 565 Reagent Dispenser can be used to maintain the liquid levels in the wash reservoirs
 - d. Place a VP 540DB Heavy Duty Blotting Station next to the daughter (recipient) plate and each wash reservoir.

B. Example of liquid sample transfer from mother plate to daughter plate:

1. Dip pins into mother plate:
 - a. Dip pins into mother plate to desired depth at maximum speed
 - b. Move stage or pin tool (at maximum speed) so the pins are just barely out of the mother plate solution.
 - c. Repeat dipping into mother plate two more times at maximum speed.
 - d. Change the speed of the pin movement to one that results in the desired transfer volume.
 - e. Raise pins out of mother plate.
2. Transfer pins to daughter plate:
 - a. Dip pins into daughter plate to desired depth at maximum speed.
 - b. Move stage or pin tool (at maximum speed) so the pins are just barely out of the daughter plate solution.
 - c. Repeat dipping into daughter plate two more times at maximum speed.

- d. Change the speed of the pin movement to the same withdrawal speed as the mother plate.
- e. Raise pins out of daughter plate.
- f. Blot the pins on the VP 540DB Blotting Station at maximum speed so the pins float up 2mm.
- g. Let the pins dwell 2 seconds on the Blotting Station.

C. Washing after each mother-to-daughter plate transfer:

1. Dip pins into first wash reservoir.
 - a. It is critical that during each dip the tip of the pin is moved up and down through the meniscus.
 - b. Move pins up and down through the meniscus 3 times.
 - c. Blot pins on VP 540DB Blotting Station.
2. Dip pins into second wash reservoir.
 - a. Move pins up and down through the meniscus 3 times.
 - b. Blot pins on VP 540DB Blotting Station.
3. Dip pins into third wash reservoir.
 - a. Move pins up and down through the meniscus 3 times.
 - b. Blot pins on VP 540DB Blotting Station.
4. Allow pins to air-dry or use a drying station as mentioned below.

D. Drying pins: If pin tool is cleaned while installed on a workstation, the pins can be air-dried for a few minutes or dried over a fan or vacuum station for one minute. Solid pins do not need to be dried with a fan or vacuum if they are blotted as the alcohol will evaporate very quickly. Slot pins will require a little longer time to dry without a fan or air movement.

III. HEAVY DUTY CLEANING FOR NEGLECTED OR INFREQUENTLY USED PIN TOOLS

A. Clean pins in 65°C Ultrasonic Cleaning Bath (Off-line)

1. Use 1:100 dilution of Micro90 Ultrasonic Cleaning Solution in an ultrasonic bath.
2. Immerse the lower 9 mm of pins in bath for 1 minute.
3. Rinse pin tips under hot, running tap water for 30 seconds.
4. Rinse pin tips with distilled H₂O squirt bottle.
 - a. If liquid has splashed on float plate, rinse float plate with distilled H₂O squirt bottle.
5. Rinse pin tips with alcohol squirt bottle.
 - a. If liquid has splashed on float plate, rinse float plate with alcohol squirt bottle.
6. Blot excess liquid with VP 522 Lint-Free Blotting Paper that is on top of a stack of paper towels. The paper towels should be stacked so that the layers are even across the area where the pin tool will be blotted.
7. Dry pin tool briefly with portable hot-air dryer or continue with "Cleaning with Pin Cleaning Solution" below.

B. Drying steps: Dry pins with portable hot-air dryer.

1. Hold pin tool in working position with pins pointing down.
2. Blow warm air, from below, up towards the pin tips.
 - a. Move air from portable dryer across all of the pin tips.
3. Gently turn pin tool upside-down with pins pointing up.
 - a. All of the floating pins should move through the float holes and rest on the top plate.
 - b. Blow warm air, from the sides, between the float plates and top plate.
4. Turn pin tool back to working position with pins pointing down.
 - a. If no pins remain stuck in the "up" position, then pin tool is ready to use.
 - b. If a few pins move slowly or remain stuck in the "up" position, repeat blowing warm air from below and sides while gently rocking the pin tool upside-down to right-side up until all pins are moving freely.

C. Clean pins in 1:3 dilution of VP 110 Pin Cleaning Solution.

1. Set up wash reservoirs as in Routine/Daily Cleaning (page 2) except that the VP 110 is diluted 1:3. Also set up an additional VP 522 Lint-Free Blotting Paper with a stack of paper towels.
2. Immerse the lower 9 mm of pins in VP 110. Move pins up and down through the meniscus 3 times.
3. Blot on VP 522 Lint-Free Blotting Paper.
 - a. Check wet spots on Blotting Paper for uniformity.
 - b. If spots are not uniform in size, continue to dip and blot a few more times until spots are uniform.
4. If spots are still not uniform after multiple dips and blots, slot pins may have dust or debris in the slots.
 - a. Rinse the pin tips under hot, running tap water.
 - b. Scrub the pin tips with VP 425 Pin Cleaning Brush or VP 426 Pin Tip Cleaning Pad.
 - c. Rinse again with hot, running tap water.
 - d. Repeat Step 3.
 - e. If necessary, examine pins under a dissecting microscope to check for lint or debris on the pins or in the slots.
5. Dip pins into VP 110 again and blot to check spot size.
6. Once spot sizes are uniform, dip again in VP 110 by moving up and down through the meniscus 3 times.
7. Immerse the lower 9 mm of pins in VP 110. Soak pins in VP 110 for 30 minutes.
8. Rinse pin tips under hot, running tap water briefly.
9. Blot the pins briefly on piece of Lint-Free Blotting.
10. Dip pins into first wash reservoir.
 - a. Move pins up and down through the meniscus 3 times.
 - b. Blot pins on VP 522 Lint-Free Blotting Paper.
11. Dip pins into second wash reservoir.
 - a. Move pins up and down through the meniscus 3 times.
 - b. Blot pins on VP 522 Lint-Free Blotting Paper.

12. Dip pins into third wash reservoir.
 - a. Move pins up and down through the meniscus 3 times.
 - b. Blot pins on VP 522 Lint-Free Blotting Paper.
13. Dry pins with portable hot-air dryer as described above.

APPENDIX 1

V&P PIN TOOL CLEANING ACCESSORIES:

- Our **VP 110 Concentrated Pin Cleaning Solution** is used to clean the pin tips, removing any build-up or residue. Dilute 1:5 before using.
- The **VP 540DB Heavy Duty Blotting System** is used to blot the pin tips after each daughter plate transfer and each wash station. It is a Nunc Omni Tray with a VP 540DB1 Super Absorbent Polypropylene Pad inside and a piece of 540D Lint-Free Blotting Paper on top of the pad. The heart of the system is the absorbent pad which will hold up to 27ml of liquid before it is saturated. The blotted liquid is transferred from the Lint-Free Paper to the Pad and a barrier holds it in the pad. This super absorbent pad means you don't have to change Blotting Stations frequently, just replace the VP 540D Lint-Free Blotting Paper at the end of each day (or as needed).
- The **VP 540D Lint-Free Blotting Paper** is cut to fit inside an Omni Tray for refills of the VP 540DB Heavy Duty Blotting System.
- The **VP 540DB1 Super Absorbent Polypropylene Pad** is cut to fit inside an Omni Tray for refills of the VP 540DB Heavy Duty Blotting System.
- The **VP 540S Polypropylene Wash Reservoir** is designed to hold the VP 426 Pin Cleaning Pad for gently cleaning debris or agar from pin tips. Includes an SBS feature on the bottom for workstation deck placement.
- The **VP 426 Pin Cleaning Pad** has soft, 4 mm long nylon bristles. It is placed in the bottom of the VP 540S Polypropylene Wash Reservoir for cleaning pin tips during the wash sequence between pin tool transfers.
- The **VP 425 Pin Cleaning Brush** is used to manually keep the replicator pins clean so they will carry the same amount of liquid each time. 2.4 cm nylon bristles.
- The **VP 425A Pin Cleaning Brush** is used to manually keep the replicator pins clean so they will carry the same amount of liquid each time. For long pins, 8.5 cm nylon bristles.
- The **VP 904 Pin Dryer** is used to speed the drying time of the pins and decrease assay cycle time.