V&P SCIENTIFIC, INC.
Innovators in Liquid Handling, Arraying and Mixing

TECHNICAL NOTE 83

PROTOCOLS FOR LIQUID SAMPLE TRANSFER WITH CYBIO ROBOT PIN TOOLS AND CLEANING CYBIO ROBOT PIN TOOLS

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

1. The first is to start with clean pins. We strongly recommend that you use our specially developed pin cleaning solution VP 110 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 each delivery to the last daughter plate and between wash solutions. Blotting greatly reduces the carry over effect and lint free blotters assure that the pins do not pick up lint which will affect the volume transferred. . We have several different formats for our lint free blotting media. Check which one fits your application at http://www.vp-scientific.com/blotting_membranes_wicking_papers.htm

3. Only 2 wash solutions are needed. The first solution can be the same solvent as used to carry the sample (DMSO, H2O, etc.) or it can be distilled H2O. Which solution you choose will be determined by the solubility of the samples being transferred. We recommend isopropanol for the second wash solution for its solvent characteristics and fast evaporation rate. We have static wash reservoirs, disposable reservoirs, static wash and blot reservoirs, flowing reservoirs and fountain reservoirs for washing pin tools. Check which one fits your application at http://www.vp-scientific.com/wash_and_blot_stations_for_robot.htm and http://www.vp-scientific.com/disposable_trays.htm

4. When dipping the pins into mother, daughter 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 re-dipping to achieve maximum effect. (Some of our customers only double dip and for their assays double dipping is sufficient - however we recommend triple dipping to be safe). See Movie of cleaning pins at Maxim Pharmaceuticals.

5. The speed of pin withdrawal from the liquid on the final dip will determine the volume transferred. See speed vs volume data. The speed of the pins during the re-dipping (washing/mixing) steps should be fast (2-8 cm/sec).

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The following outline is a detailed procedure and recommended speeds.

Liquid-to-Liquid Transfer - One Mother Plate to One Daughter Plate

1. Initial Wash With V&P Pin Cleaning Solution (VP 110)

- Prepare three, VP 540 Wash Reservoirs
- Place a small amount of silicone stopcock grease on each of the VP 540 locating pins so liquids don't wick around the locating pin to the VP 540AC Lint Free Blotting Media.
- Fill first VP 540 Wash Reservoir with 195 mls of V&P Pin Cleaning Solution
- Fill second VP 540 Wash Reservoir with 200 mls of distilled water
- Fill third VP 540 Wash Reservoir with 205 mls of isopropanol
- Place VP 540A Support and VP 540AC lint free Blotting Media on each Wash Reservoir
- Position pin tool in V&P Pin Cleaning Solution to get maximum coverage of pins
- Hold in this position for 30 seconds
- Move the stage or pins so the pins are just barely out of the cleaning solution
- Repeat dunking 2 more times at a speed of ~6.5 cm/sec
- Change the speed of the pin movement to approximately 0.7cm/sec
- Raise the pins above the VP 540AC Blotting Media
- Position the pins so they are over the web in the VP 540AC Blotting Media
- Touch the pins at ~6.5 cm/sec onto the Blotting Media so the pins float up 2 mm
- Let the pin dwell 2 seconds on the Blotting Media
- Move the pins to the VP 540 Wash Reservoir filled with 200 ml of distilled water
- Position pin tool in distilled water to get maximum coverage of pins
- Position pin tool in distilled water to get maximum coverage of pins
- Move the stage or pins so the pins are just barely out of the water
- Move the stage or pins so the pins are just barely out of the water
- Repeat dunking 2 more times at ~6.5 cm/sec
- Change the speed of the pin movement to approximately 0.7cm/sec
- Raise the pins above the VP 540AC Blotting Media
- Position the pins so they are over the web in the VP 540AC Blotting Media

- Touch the pins at 6.5 cm/sec onto the Blotting Media so the pins float up 2 mm
- Let the pins dwell 2 seconds on the Blotting Media
- Move the pins to a VP 540 Wash Reservoir filled with 205 mls isopropanol
- Position pin tool in the isopropanol to get maximum coverage of pins
- Move the stage or pins so the pins are just barely out of the isopropanol
- Repeat dunking 2 more times at ~6.5 cm/sec
- Change the speed of the pin movement to approximately 0.7cm/sec
- Raise the pins above the VP 540AC Blotting Media
- Position the pins so they are over the web in the VP 540AC Blotting Media
- Touch the pins at ~6.5 cm/sec onto the Blotting Media so the pins float up 2 mm
- Let the pins dwell 2 seconds on Blotting Media
- The pins are now ready to be used

2. Liquid Transfer

- Prepare two VP 540 Wash Reservoirs
- Fill first VP 540 Wash Reservoir with 200 mls of DMSO
- Fill second VP 540 Wash Reservoir with 205 mls of isopropanol
- Place VP 540A Support and VP 540AC Blotting Media on each Wash Reservoir
- Place pins in mother plate to desired depth at ~6.5 cm/sec
- Move the stage or pins so the pins are just barely out of the mother plate solution
- Repeat dunking 2 more times at a speed of ~6.5 cm/sec
- Change the speed of the pin movement to around 0.7cm/sec
- Raise the pins out of the mother plate
- Transfer the pins to a daughter plate and change the speed to ~6.5 cm/sec
- Place the pins in the daughter plate to the desired depth (greater than mother plate)
- Move the stage or pins so the pins are just barely out of the daughter plate solution
- Repeat dunking 2 more times at a speed of 6.5 cm/sec

- Change the speed of the pin movement to ~0.7cm/sec
- Raise the pins out of the daughter plate
- Position the pins over a VP 540 Wash Reservoir with 200 ml of DMSO
- Change the speed to 6.5 cm/sec and blot the pins on the web of the VP 540AC
- Position the pins so they are over the holes in the VP 540AC
- Lower the pins into the DMSO to a depth greater than the daughter plate
- Move the pins so the pins are just barely out of the DMSO
- Repeat dunking 2 more times at ~6.5 cm/sec
- Change the speed of the pin movement to ~0.7cm/sec
- Raise the pins above the VP 540AC Blotting Media
- Position the pins so they are over the web in the VP 540AC Blotting Media
- Touch the pins at ~6.5 cm/sec onto the Blotting Media so the pins float up 2 mm
- Let the pin dwell 2 seconds on the Blotting Media
- Move the pins to a VP 540 Wash Reservoir filled with 205 mls isopropanol
- Position pin tool in the isopropanol to get maximum coverage of pin
- Move pins so the pins are just barely out of the isopropanol
- Repeat dunking 2 more times at ~6.5 cm/sec
- Change the speed of the pin movement to ~0.7cm/sec
- Raise the pins above the VP 540AC Blotting Media
- Position the pins so they are over the web in the VP 540AC Blotting Media
- Touch the pins at ~6.5 cm/sec onto the Blotting Media so the pins float up 2 mm
- Let the pins dwell 2 seconds on Blotting Media
- The pins are now ready for next mother plate

Complete pin tool procedures and program downloads for Cybi Well Robot systems are available in the Technical Notes area of the Website: (http://www.vp-scientific.com/technotes.htm).