

7: Getting the Most Out of Your Hydra II Microdispenser

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Basics of Liquid Handling With the Hydra II Microdispenser

To use your Hydra II microdispenser effectively, you need to first understand some of the basics of its operation. A discussion of (1) **Aspirate/Dispense modes**, (2) **priming syringes**, and (3) **setting dispense heights** is provided here to give you basic information on the fundamental operations of the Hydra II microdispenser.

Two Aspirate/Dispense Modes

Different liquid handling applications require different Aspirate/Dispense modes.

The Hydra II microdispenser has two Aspirate/Dispense modes:

- Aspirate with Overstroke/Dispense Specific
- Aspirate with Pre Air Gap/Dispense All

Some of the terms used in the Hydra II EPROM-file display differ from the ones used in the ControlMate software. Please see the following table.

Table 7-1: Related Terms in ControlMate Software for Hydra II & in Hydra II EPROM-File Display

ControlMate Term	EPROM-File Term
Pre Air Gap	Air
Dispense All	Empty
Dispense Specific	Dispense
Overstroke	Prime

The rest of this discussion about the two modes will use the ControlMate terms *unless* a distinction must be made between the two types of terms.

When is Aspirate with Overstroke/Dispense Specific the right mode to use?

The Aspirate with Overstroke/Dispense Specific mode is best used for procedures that require:

- Highest accuracy for submicroliter and small-volume dispenses
- Serial dispensing
- Bulk transfers

Serial dispensing using this mode is one of the most efficient means of transferring microliter volumes into any number of 96-, 384-, or 1536-well plates. (Note that your Hydra II system must be equipped with the X/Y Plate Stage and the accompanying ControlMate software to dispense into 1536-well plates.)

This mode allows the most accurate method of repetitive dispensing and also allows dispensing to multiple plates without the need to refill the syringes.

How is Overstroke used in Aspirate with Overstroke/Dispense Specific mode?

The Aspirate with Overstroke command obtains the desired, programmed volume in the syringes in this way:

1. It first draws in the specified Aspirate volume amount.
2. Then it overshoots that volume by drawing in an additional Overstroke volume.



Note: The Overstroke volume can be reset on the Set Device and Pipettors Options screen under the Add-Ins menu. The Prime volume in the EPROM files is pre-set to 5% of total syringe volume and is not a user-editable value.

3. Finally it pushes the plungers back down into the syringes, dispensing the overshoot volume into the source reservoir and leaving the desired amount in the syringes with no excess.

The actions of the Aspirate with Overstroke command force the syringes against the lower holding plate to seat them in preparation for dispensing. The syringes have been “auto-primed” (primed by default) one time. Apogent Discoveries recommends that you follow this default priming with 3 to 5 more manual primes just before the Dispense operation is performed. See page 7-8 for more information about priming syringes.

After the Aspirate operation has finished, you use the Dispense command to dispense the programmed volume into the receiving plate. When you select the Specific option in the Dispense command, the command empties just the volume that you have set in your file as the Dispense Volume, rather than the entire contents of the syringe.

How do I program a file to use the Aspirate with Overstroke/Dispense Specific mode and then run the file?

The following paragraphs provide a basic description of how to program a file in this mode and then run the file. For detailed information about creating, changing, and saving the files you will use to run protocols on the Hydra II microdispenser, see Chapter 3 if you are using Manual Operation or Chapter 4 if you are using Automated Operation.

In Manual Operation:

To program a Hydra II EPROM file to use the Aspirate with Overstroke/Dispense Specific* mode, use the following procedure. (*Note: In the EPROM files, the “Aspirate with Overstroke/Dispense Specific” mode is actually “Aspirate with Prime/Dispense.”)

1. In your chosen Hydra II file, set the specified Dispense and Aspirate parameters as follows:

Screen	Parameter	Value
Dispense	Vol	Specific incremental (partial) dispense amount required by your application.
Aspirate	Prime*	ON
	Vol	Aspirate volume sufficient to provide as many of your dispense volumes as the syringes will hold, allowing space in the syringe for the Prime volume*.
	Air	0 (zero)
*Prime volume is pre-set to 5% of the total syringe volume and is not a user-editable value.		

2. Set the other file parameters (for example, Table Height and Speed that are correctly calibrated for your application and labware) in the file.

To run a Hydra II EPROM file in the Aspirate with Overstroke/Dispense Specific mode:


1. Access the Ready Mode screen for the file.
2. Put an empty reservoir on the tray table/stage and press **Empty** to empty the syringes.
3. Replace the reservoir with the source plate containing the sample.
4. **Press Aspr to aspirate** the **Vol** and the **Prime** volume into the syringes. (Vol should be large enough to allow you to dispense the Dispense Vol amount into as many of your receiving microplates as possible while still leaving space in the syringe for the Prime volume.)
5. Remove the source plate and place the destination plate on the tray table/stage.
6. **Press Dispense to dispense Vol** into the destination plate.
7. Continue to remove and replace destination plates and to press Dispense until the sample volume in the syringes is too low to provide more dispenses.
8. Repeat Step 3 through Step 7 as many times as needed for multiple source and destination plates.
9. When you are finished dispensing, press **Empty** to empty any remaining sample volume back into the source plate or into a waste reservoir.

In Automated Operation:


To program a sequence file to use the Aspirate with Overstroke/Dispense Specific mode, use the following procedure:

1. In your sequence file, set the specified Aspirate and Dispense parameters as follows:

Command	Parameter	Value
Aspirate	Overstroke*	Option is selected.
	Aspirate volume	Aspirate volume sufficient to provide as many of your dispense volumes as the syringes will hold, with just enough space in the syringe for the Overstroke volume.*
Dispense	Specific	Option is selected.
	Volume	Specific incremental (partial) dispense amount required by your application.
*Overstroke volume is set on the Set Device and Pipettor Options screen under the Add-Ins menu. If you need to fine-tune the value for your applications, you can reset it there.		

2. Set other command parameters in the file as needed.
3. Click the **Validate File** icon  in the **Toolbar**. If any errors are listed, correct them and then re-validate the file.

To run a sequence file in the Aspirate with Overstroke/Dispense Specific mode:

1. Access a Hydra II EPROM file, and check that the Empty (Table) height is set to a safe height.
2. Put an empty reservoir on the tray table or on the stage in the source nest under the dispensing head and press **Empty** to empty the syringes.
3. Replace the reservoir with the source plate containing the sample.
4. Access the sequence file in the ControlMate software.
5. If the system is a Hydra II with X/Y Plate Stage system, place the first destination plate in the destination nest.
6. Click the **Run**  icon on the **Toolbar**.
7. After the Aspirate with Overstroke command has executed: On the Hydra II base-unit system or Hydra II with Automatic Syringe Wash Module, remove the source plate and place the destination plate on the tray table.
8. Continue to remove and replace destination plates as your sequence file requires.
9. Repeat Step 4 through Step 8 as many times as needed for multiple source and destination plates.

10. When dispensing is completed, press **Empty** on the Hydra II microdispenser to empty any remaining sample volume back into the source plate or into a waste reservoir.

When is Aspirate with Pre Air Gap/Dispense All the right mode to use?

The Aspirate with Pre Air Gap/Dispense All mode is best used for procedures in which:

- Source plates have minimal sample volumes.
- Residual sample cannot be returned to the source plate after the transfer.
- Multiple samples are pooled.
- Individual samples of microliter volumes are transferred.

Use of this mode is necessary when there is insufficient sample to allow for the overshoot volume that occurs with the Aspirate with Overstroke command. Sample volumes of as little as 1.0 μ L have been transferred using the Aspirate with Pre Air Gap/Dispense All mode.

Aspirate with Pre Air Gap/Dispense All mode is also preferred if the user does not want to draw excess sample into the syringes only to return the remainder of the sample to the sample plate after the transfer.

When pooling multiple samples, you use the Aspirate command to draw first an air gap (see Table 7-2 on page 7-10 for recommended air-gap volumes) and then the samples into the syringes. Then you use the Dispense All command to deliver the pooled samples and the air gap into the final receiving plate.

Microliter volumes of individual samples can also be transferred using the Aspirate with Pre Air Gap/Dispense All mode. Here, as with pooling, you first draw an air gap (see page 7-9 for recommended air-gap volumes) into the syringes using Aspirate, and then you use Aspirate to draw the single volumes to be transferred into the syringes. Finally, you use the Dispense All command to deliver the entire contents of the syringes, including the sample volumes and the air gap, into the receiving plate.

Why are air gaps used in the Aspirate with Pre Air Gap/Dispense All mode?

Aspirating an air gap into the syringes at the beginning of a procedure in the Aspirate with Pre Air Gap/Dispense All mode increases accuracy in dispensing individual small volumes and helps to force all of the sample volume out of the syringes during the dispense. For details about using an air gap, see “Priming Syringes in Aspirate with Pre Air Gap/Dispense All Mode” on page 7-9.

How do I program a file to use the Aspirate with Pre Air Gap/Dispense All mode and then run the file?

The following paragraphs provide a basic description of how to program a file in this mode and then run the file. For detailed information about creating, changing, and saving the files you will use to run protocols on the Hydra II microdispenser, see Chapter 3 if you are using Manual Operation or Chapter 4 if you are using Automated Operation.

In Manual Operation:

To program a Hydra II EPROM file to use the Aspirate with Pre Air Gap/Dispense All mode, use the following procedure:

1. In your chosen Hydra II file, set the specified parameters as follows:

Screen	Parameter	Value
Aspirate	Prime	OFF
	Vol	Total sample volume
	Air	Recommended air gap for your system (See Table 7-2 on page 7-10.)

2. Set the other file parameters (for example, Table Height and Speed that are correctly calibrated for your application and labware) in the file.

To run a Hydra II EPROM file in the Aspirate with Pre Air Gap/Dispense All mode:


1. Access the Ready Mode screen for the file.
2. Put an empty reservoir on the tray table/stage and press **Empty** to empty the syringes.
3. Replace the reservoir with the source plate containing the sample.
4. **Press Aspr to aspirate Vol and Air** into the syringes.
5. Remove the source plate and place the destination plate on the tray table/stage in the nest under the dispensing head.
6. **Press Empty to dispense the total syringe contents** into the destination plate.

In Automated Operation:


To program a sequence file to use the Aspirate with Pre Air Gap/Dispense All mode, use the following procedure:

1. In your sequence file, set the specified Dispense and Aspirate parameters as follows:

Command	Parameter	Value
Dispense	All	(Option is selected.)
Aspirate	Pre air gap	(Option is selected.)
	Pre air gap volume	Recommended air-gap volume for syringe size (See Table 7-2 on page 7-10.)
	Aspirate volume	Total sample volume, or as much of sample as will fit in syringe with air gap

- Set other command parameters in the file as needed. If your system is a Hydra II with X/Y Plate Stage system, include **Move** commands that move the source and destination nests under the dispensing head.
- Click the **Validate File** icon  in the **Toolbar**. If any errors are listed, correct them and then re-validate the file.

To run a sequence file in the Aspirate with Pre Air Gap/Dispense All mode:

- Access a Hydra II EPROM file, and check that the **Empty** (Table) height is set to a safe height.
- Put an empty reservoir on the tray table/stage in the nest under the dispensing head and press **Empty** to empty the syringes.
- Replace the reservoir with the source plate containing the sample.
- Access the sequence file in the ControlMate software.
- On the Hydra II with X/Y Plate Stage, place the destination plate in the destination nest.
- Click the **Run**  icon on the **Toolbar**.
- On the Hydra II base-unit system or Hydra II with Automatic Syringe Wash Module: Follow software-prompt instructions to remove the source plate and insert the destination plate.

Priming Syringes

After the Hydra syringes have been filled with the solution to be dispensed, the syringes must be “primed” to ensure accuracy and precision of the operation. To explain the reasoning behind the need to prime the syringes, a short discussion of the Hydra II design is presented here. There is a slight amount of movement (referred to as “lash”) of the glass syringes that is designed into the Hydra II micro-dispenser such that at the beginning of a Dispense or Aspirate step, the syringe barrels move up or down together with the plungers until they contact the upper or lower holding plate. (Holding plates pro-

vide vertical and horizontal positioning of the syringes within the head unit of the microdispenser.) During this short interval of “lash movement” the plunger does not move inside the barrel of the syringe, so no sample is dispensed or aspirated. After the syringe barrels have been seated against the holding plate, the plunger begins to move within the syringe and aspirating or dispensing of sample begins.

The way to account for the effects of lash is to “**prime**” the syringes. The following two subsections tell you how to prime the syringes, first for the Aspirate with Overstroke/Dispense Specific mode and then for the Aspirate with Pre Air Gap/Dispense All mode.

Priming Syringes in Aspirate with Overstroke/Dispense Specific Mode

Aspirate with Overstroke/Dispense Specific mode uses the Aspirate with Overstroke option to fill the syringes for a dispense operation. The Overstroke action provides initial priming. The microdispenser draws the desired amount of liquid into the syringes, overshoots this volume by the specified Overstroke volume (typically an additional 5–50 μ L), and then dispenses the extra volume back into the reagent reservoir, leaving the desired volume in the syringes. (The necessary amount of Overstroke volume depends on syringe size. In ControlMate software, it is set by the user on the Set Device and Pipettors Option screen under the Add-Ins menu. In the Hydra II EPROM files, the (Prime) value is set to 5% of total syringe volume and is not user-editable.) This default priming forces the syringes against the lower holding plate to seat them in preparation for dispensing. Apogent Discoveries recommends that immediately before the dispense is to be performed you supplement the default priming by dispensing the desired volume back into the sample reservoir 3–5 times. At this point the syringes will be seated firmly against the bottom holding plate so that any further downward movement of the plungers will result in the precise volume of fluid being dispensed from the syringes. The dispense operation should be carried out immediately after the last priming.

Priming Syringes in Aspirate with Pre Air Gap/Dispense All Mode

Aspirate with Pre Air Gap/Dispense All mode requires a method to account for lash other than the one used in Aspirate with Overstroke/Dispense Specific mode. In an aspirate-transfer, the Aspirate command (with no Overstroke) is used to draw sample into the syringes. When an Aspirate command is executed, the plungers are immediately raised, resulting in: first, the joint upward movement of the syringes and plungers; second, seating of the syringes against the upper holding plate of the head; and finally, aspirating of liquid into the syringe needles. The result is less than the desired amount drawn

into the syringes. To prevent this error, program your file so that an initial air gap (see Table 7-2, below) is drawn into the syringes *before* the Aspirate command is used to draw sample into the syringes. Here are recommended air gap volumes:

Table 7-2: Recommended Air Gap Volumes

Syringe Size	Recommended Air Gap Volume
100 μ L, 290 μ L	10 μ L
580 μ L	50 μ L
1mL	100 μ L

Drawing air into the syringes will seat them firmly against the top holding plate in preparation for aspirating.

Setting Dispense Heights

Dispense heights are set on the Hydra II microdispenser by raising and lowering the tray table/stage. Different dispense heights are required for different types of dispense operations. If you are dispensing very small volumes onto dry plates, for example, you will set the height differently than you would for a larger-volume dispense into a plate that contained buffer.

The following figures illustrate various dispense scenarios, with dispenses into wet and dry plates and with differences in dispense volumes. The information presented here should help you determine the optimum dispense height for your applications.

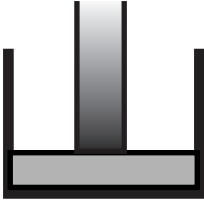
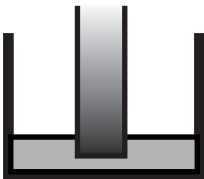
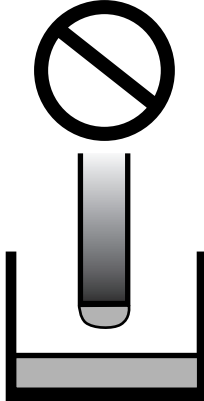


Note: For many operations you need to set the tray table/stage height to a near-bottom-of-the-well position. For instructions on how to do this, see “Set the tray table/stage height” on page 3-11.

Dispensing Into Wet Plates

Figure 7-1 through Figure 7-3 illustrate dispenses into wet plates. They show three dispenses with the needle tips at slightly different heights. (They also illustrate that dispense height can vary considerably more when dispensing into a wet plate than onto a dry plate and still result in a successful dispense.)

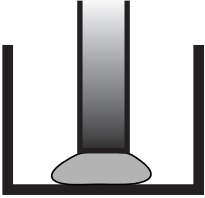



Table 7-3: Dispensing Into Wet Plates

	<p>Figure 7-1: Dispense into wet plate, with needle tip just touching solution Figure 7-1 shows the needle tip just touching the solution already in the well. This dispense height will allow effective delivery of sample to the well while minimizing the exposure of the needle tip to the solution in the well.</p>
	<p>Figure 7-2: Dispense into wet plate, with needle tip submerged in solution Figure 7-2 shows the needle tip submerged in solution. The sample will be delivered successfully into the solution in the well.</p>
	<p>Figure 7-3: Dispense into wet plate, with needle tip above solution Figure 7-3 shows the needle tip above the solution, with the drop on the needle tip not touching the solution. No sample is delivered during this dispense. The drop will remain on the needle tip.</p>

Dispensing Onto Dry Plates

Figure 7-4 through Figure 7-7 illustrate dispenses onto dry plates.

Table 7-4: Dispensing Onto Dry Plates

	<p>Figure 7-4: 10µL dispense onto dry plate—drop fills gap between needle tip and well bottom. Touch-off occurs.</p> <p>Figure 7-4 shows a 10µL dispense onto a dry plate. For this type of dispense, you set the needle tips so that they are close to but not touching the bottom of the well — a “near bottom” position. The drop on the tip of the needle fills the gap between the needle tip and the bottom of the well. When the needle is retracted, the drop will “touch off” onto the well bottom. The dispense is successful.</p>
	<p>Figure 7-5: 1µL dispense onto dry plate—drop unable to bridge gap between needle tip and well bottom. Drop remains attached to tip.</p> <p>Figure 7-5 shows a needle tip at the same dispense height as the one in Figure 7-4. For this dispense, however, the dispense amount is very small—1µL. As illustrated, the drop is not large enough to fill the gap between the needle tip and the bottom of the well, and the drop remains attached to the needle tip, so there is no “touch off.” The dispense is unsuccessful.</p> <p>Solution: Reset the Dispense height parameter so the needle tips are touching the well bottom during the dispense.</p>
	<p>Figure 7-6: 1µL dispense onto dry plate—needle tip touches plate bottom and drop dispenses around tip onto well bottom.</p> <p>Figure 7-6 shows the needle tip at a dispense height that just touches the tip to the bottom of the well. To set this height, move the tray table/stage up so that you can feel that some of the needle tips are just touching the well bottom, and then move the tray table/stage up one or two more clicks. You can check whether needles are touching the plate by trying to lift the front of the plate with your forefinger. If the plate does not lift, the needle tips are touching the plate. This figure shows the same 1µL dispense amount as in Figure 7-5. This time, the dispense is successful because the drop squeezes out from the needle tip onto the bottom of the well and touches off as the tray table/stage drops down. Because the amount of dispense is so small (<3µL), pressure does not build back up into the syringe even though the needle tip is pressing down onto the plate.</p>
	<p>Figure 7-7: 10µL dispense onto dry plate; needle tip touches plate bottom. Drop dispenses around tip onto well bottom but cannot dispense quickly enough to prevent pressure buildup.</p> <p>Figure 7-7 shows the needle tip in the same position as in Figure 7-6, with the tip touching the bottom of the well. In this instance the dispense amount is 10µL, and the needle cannot dispense the amount quickly enough to keep the pressure from building back up into the syringe. This pressure buildup is likely to damage the syringe. Solution: Reset the Dispense height parameter so the needle tips are not touching the well bottom of the plate.</p>

Caution: In general, do not allow the needles to contact the bottom of any plate, tube, reservoir, tray, or wash basin. The only time the needles need to touch the bottom of the plate is when they are dispensing volumes of 3 μ L or less onto a dry plate (as shown in Figure 7-6). For dispense volumes greater than 3 μ L, the size of the droplet formed on the needle tip will be large enough to bridge the gap between the tip of the needle and the bottom of the plate. In such cases, set the dispense height at two to three clicks back from touching the plate. If the needles touch the plate during a dispense with volume greater than 3 μ L, backpressure will build up in the syringes (as shown in Figure 7-7), because the sample cannot exit the needles as fast as the syringe plunger is pushing the sample out. That backpressure can damage the syringes. **If a syringe is damaged during operation, turn off power to the unit immediately by pressing the Emergency Shutoff Switch.**



For More Detailed Information About Hydra Microdispenser Operation

For more detailed information about the microdispenser's operation, see the Hydra Technical Data page on the ApogentDiscoveries website at <http://www.apogentdiscoveries.com/>.

Preventing Contamination Between Sample Preparations

To prevent contamination of archival source plates and sequential destination plates, use the proper cleaning protocol to clean the syringes between samples. See Chapter 6 for recommended wash procedures.

Preventing Carry-over Contamination When Preparing PCR Reactions

ApogentDiscoveries' laboratories tested the following cleaning protocols and verified that these protocols prevented carry-over contamination when using the Hydra II microdispenser to prepare PCR reactions:

If the samples include nucleic acids, wash the syringes with a 2% solution of household bleach, 3 wash cycles. (Bleach degrades nucleic acids. To prolong syringe and tip life, ANY concentration of nucleic acid is best handled with a wash that includes bleach solution.) Adding 2mL of household bleach to 98mL water makes a 2% bleach solution. Because household bleach is typically 5.25% sodium hypochlorite, the dilute 2% bleach solution can also be defined as 0.1% sodium hypochlorite. After the bleach wash has finished, immediately wash the syringes 3 wash cycles with dH₂O to remove the bleach. Ensure that the volume of dH₂O is greater than the volume of the bleach solution so the outsides of the needles are rinsed free of any chlorine-bleach residue.

For a more detailed discussion of preventing carry-over contamination when preparing PCR reactions, see the Application Note “Preparation of PCR Reactions with the Hydra[®]-96: Preventing Carry-over Contamination Between Samples” see the Hydra Technical Data page on the ApogentDiscoveries website at <http://www.apogentdiscoveries.com/>.

Preventing Carry-over Contamination in Sequencing Reactions

Guidelines for washing DNA template from syringes when preparing sequencing reactions follow those used for PCR reactions. Users may find that less aggressive washing is required for sequencing-reactions preparation than for PCR-reactions preparation due to the linear amplification of template in sequencing reactions versus the exponential amplification during PCR.

Preventing Carry-over Contamination for DMSO-based Samples

To prevent carry-over contamination when you are using the Hydra II microdispenser to dispense DMSO-based samples:

1. Wash the syringes after each use with 3 wash cycles of DMSO.
2. Wash them with 3 wash cycles of methanol:acetone (1:1 v/v).
3. Wash them with 1 wash cycle of methanol or ethanol.
4. Wash them with 3 wash cycles of dH₂O.

Preventing Cross-Contamination: Dispensing Onto the Sidewalls of Wells in CyclePlate-ET Plates

To dispense droplets of different solutions into a microplate well without touching the needle tips or the solution to be dispensed to the current contents of the wells, set up the instrument to dispense onto the sidewalls rather than into the center of the well. The following procedure describes how to dispense solutions onto the sidewalls of the 96 wells in a CyclePlate-96ET without touching any other solution currently found in the wells (you can use the same procedure with CyclePlate-192 and -384 microplates).



Note: In Manual Operation, this procedure requires use of the split-well manually operated plate positioner, the Hydra-96 Plate Positioner for CyclePlate-384 and -192ET, ApogentDiscoveries Catalog No. 1029-41-0.

In Manual Operation:

1. Place a CyclePlate-96 ET PCR plate in the split-well Hydra-96 Plate Positioner for CyclePlate-384 and -192ET (manually operated plate positioner).
2. Transfer the assembly onto the tray table of the Hydra II 96 microdispenser.

3. **Set the Dispense (Table) height** so the needle tips are just touching the sides of the conical wells above the expected level of solution when the sample is transferred.
4. Set all other file parameters as needed.
5. Remove the microplate and replace it with an empty reservoir identical to that containing your sample solution. Set the Aspirate Volume, Aspirate (Table) height, and Dispense (Table) height parameters accordingly.
6. Remove the empty reservoir and replace it with the source reservoir containing your sample(s).
7. Fill the syringes with the sample solution and then remove the reservoir.
8. Remove the source reservoir and position your first destination CyclePlate-96 ET microplate in the manual plate positioner. Place the positioner with the CyclePlate on the tray table.
9. Dispense the solution into the microplate. The droplet should just touch off onto the side of the well.
10. Repeat Step 8 and Step 9 for as many microplates as you want to fill with the sample solution.
11. Clean the syringes as needed between multiple samples.
12. Repeat steps 1 – 11 for each new solution sample plate, advancing the split-well positioner to the next position each time that you change solutions. You can perform this procedure for four separate sample or reagent solutions so that each one is placed on a different side of the well.
13. **Centrifuge** the destination plate after the final solution addition to pool all the reagents in the bottoms of the wells.



Figure 7-8 and Figure 7-9 on page 7-16 illustrate use of the Hydra-96 Plate Positioner for CyclePlate-384 and -192 (a manually operated, split-well plate positioner, ApogentDiscoveries Catalog No. 1029-41-0) to dispense solutions onto the sidewalls of CyclePlate wells.

In Automated Operation:

On the Hydra II with X/Y Plate Stage system, use tip/well offset settings on the Move to Position command screen to position the depth and X-Y placement of the needle tips in relation to the centers of the wells.

On the Hydra II base-unit system or Hydra II with Automatic Syringe Wash Module system, use the manual plate positioners as described previously.

Table 7-5: Dispensing Solutions Onto Sidewalls of CyclePlate Microplate Wells

	<p>Figure 7-8: Needle Position for PCR Plate on Tray Table When a CyclePlate-96ET plate is used on the Hydra II microdispenser tray table, the needle tip will dispense into the center of the well, as shown in Figure 7-8.</p>
	<p>Figure 7-9: Needle Position for PCR Plate on Plate Positioner Use of the Hydra-96 Plate Positioner for CyclePlate-384 and -192 plates allows you to dispense solution onto the side of a CyclePlate well rather than into the center. This technique guards against cross-contamination of samples by allowing solution to be added to a well without touching any sample currently in the plate. Figure 7-9 shows the needle position.</p>

Using the Microdispenser in Cell Culture Applications

Some laboratories have reported success in using the Hydra II microdispenser to transfer cells and cell culture media. To create the required sterile environment, take the following steps:

1. Place the Hydra II microdispenser in a laminar flow tissue culture hood. Best results will be achieved if the unit is left in the hood. An initial wipedown of the Hydra II microdispenser with ethanol and paper towels is recommended. You can wipe down the instrument periodically whenever you similarly clean the inside of the culture hood.



Note: The Hydra II microdispenser can be kept in a hood with the UV (ultraviolet) light on for extended periods. However, the plastic components on the outside of the Hydra II case (that is, the switches, the cover over the display, and the data-port connectors) might be damaged after prolonged UV exposure. If those parts become corroded, you will need to replace them.

Caution: Do NOT pass syringe needles through the flame of a Bunsen burner.



2. You can sterilize the syringes initially if you remove them from the Hydra II microdispenser and soak them in a 100% ethanol solution. If you do this, you must ensure that each syringe remains paired with its original plunger tip throughout the lifespan of the plunger tip. (See Table 6-4 on page 6-12 for Teflon tip replacement guidelines.)
3. A less laborious approach would be simply to fill the syringes with a 100% ethanol solution, leave the reservoir with the ethanol solution on the tray table/stage, and leave the tray table/stage in the up position so that the needle tips are submerged in the ethanol solution. To leave the syringes soaking in the ethanol solution: Place the reservoir containing the ethanol solution on the tray table/stage. In an EPROM file, set **Vol** to full volume. Before you press the **Aspr** button, position yourself so you can easily reach the power switch on the back of the microdispenser. Press **Aspr**, and when the syringes are full and the tray table/stage is up so that the needle tips are submerged by at least 2cm, press the power switch to **OFF**. Now both the needle tips and the interior of the syringe barrels will be bathed in the ethanol solution during the storage interval. After the syringes are soaked, the ethanol should be discarded and the syringes rinsed with 3 wash cycles of sterile dH₂O.
4. When the Hydra II microdispenser is used to transfer culture media or media containing suspended cells, keep in mind that ethanol may lyse cells. To minimize the chances of cell lysis inside the syringes: Wash the syringes between uses of different media or reagents with 3 wash cycles of ethanol and as many as 9 wash cycles of autoclaved dH₂O. **Use fresh sterile water for every 3 wash cycles.**
5. To further ensure that the syringes are clean, you can wash the syringes with a nonionic detergent solution such as Coulter® Clenz (Beckman Coulter cat. #s 8546929–31). See “Washing Syringes” on page 6-3 for information on cleaning with detergent solution. If you use a detergent wash, **ensure that all residual detergent is removed** by washing the syringes with multiple water washes after the detergent wash.

Creating Dot Blots and Membrane Arrays With the Vacuum Manifold

The Hydra II 96 base-unit system and Hydra II 384 base-unit system microdispensers can be used to prepare dot blots and membrane arrays of various densities. To prepare a simple dot blot, put several pieces (approximately 5) of filter paper on the tray table of the microdispenser and lay a membrane (dry or pre-wetted) on top of the

paper. You can also place the filter paper and membrane in a Multi-Blot® tray (Catalog Nos. 1039-50-0 [standard] and 1039-51-0 [sterile]) for this purpose if you wish. Set the dispense height so that the needles will press the membrane down onto the filter paper but will not penetrate it when sample solutions are dispensed.

For creating arrays of densities greater than 96 samples on the membrane, with the samples placed within the spacing of a standard microplate, Apogent Discoveries recommends using our vacuum manifold (Catalog No. 1029-42-0) because of the following advantages:

- The membrane is anchored firmly by the vacuum, so it will not shift during multiple spottings.
- The vacuum manifold ensures a uniform vacuum across the membrane and helps limit diffusion of the sample as it is being applied to the membrane, giving a more concentrated sample and a smaller spot size that allows higher density arrays.

To use the vacuum manifold, follow these steps:

1. Place the manifold on the Hydra II microdispenser tray table and connect it to a vacuum source.
2. Place a dry or pre-wetted membrane on the manifold. Ensure that the membrane covers the top of the manifold completely but is not larger than the top of the manifold.
3. Adjust the Dispense (**Table**) height parameter so that the needles hover above the membrane by approximately 1mm. This height setting will allow effective touch off during dispense while avoiding penetration of the membrane by the needle tips.
4. Run the Hydra II microdispenser dispense protocol to spot solutions containing target molecules onto the membrane.

With the Hydra II 96 base-unit system you can use the standard plate positioner (see “Using the Manually Operated Plate Positioners for the Hydra II 96 Microdispenser” on page 7-19) with the vacuum manifold to create membrane arrays with a density of 384 samples. Figure 7-10 shows the two manually operated plate positioners and the vacuum manifold.

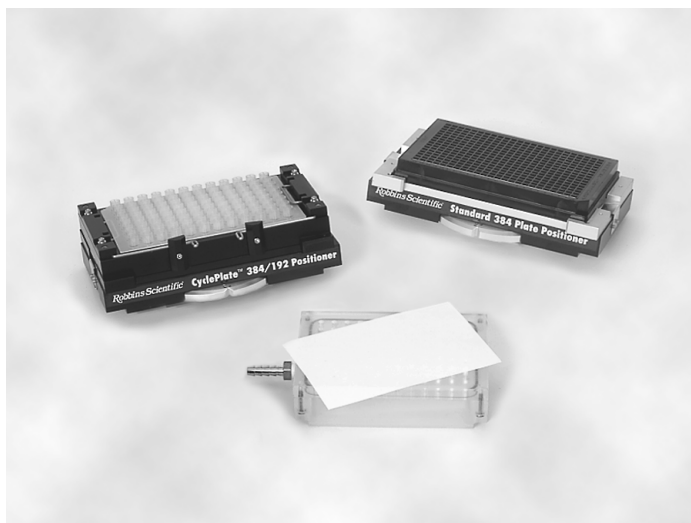


Figure 7-10: Manually Operated Plate Positioners and Vacuum Manifold

Note: With the Hydra II with X/Y Plate Stage system, you can also use the manifold to create membrane arrays with densities of 1536 to 9600 samples.



For more information on the vacuum manifold and other accessories for the Hydra II microdispenser, including the manual plate positioners, and on MultiBlot trays, see the ApogentDiscoveries website at <http://www.apogentdiscoveries.com/>.

Using the Wash Operation as a Mix Operation

In Manual Operation mode, you can use the Wash operation to mix multiple solutions added sequentially to the wells of a microplate. (In Automated Operation mode, use the Mix command. See page 4-65 for information about the Mix command.)

Dispense each of the solutions you will mix into the same microplate, one after the other. Set the Wash (Table) height close to the bottom of the plate and the wash volume (Wash Vol) to draw up less than the total volume of the combined solutions into the syringes. Push the Wash button. All the combined solutions (less a small amount) will be aspirated and then emptied back into the microplate.

When you set up your file parameters for this operation, the Wash (Table) height parameter must be set so the needle tips are submerged below the top of the combined solutions and so that no air is drawn into the syringes when the wash volume is drawn up from the microplate.

Using the Manually Operated Plate Positioners for the Hydra II 96 Microdispenser

To accommodate the well spacing of different 96-well and 384-well microplates, ApogentDiscoveries offers two manually operated plate

positioners for the Hydra II 96 microdispenser for dispensing into 384-well plates:

- Hydra-96 Plate Positioner for Standard 384 Well Plates (Catalog #1029-41-5)
- Hydra-96 Plate Positioner for CyclePlate®-384 and -192 (Catalog #1029-41-0)

Figure 7-10 on page 7-19 shows the manually operated plate positioners.

The positioner for standard 384-well plates is based on the 4.5mm center-to-center spacing found in 384-well plates that adhere to the Society for Biomolecular Screening (SBS) standards for size and shape. When the positioner is used to create membrane arrays of 384 samples, the spots on the membrane will also have a spacing of 4.5mm.

The “split-well” positioner is needed when using the Hydra II microdispenser to transfer liquid to and from CyclePlate®-192ET and -384ET microplates. You can also use it with the CyclePlate-96, -192, or -384 ET plates to dispense solutions onto the sidewalls of the wells rather than into the centers of the wells. See page 7-14 for information about dispensing onto sidewalls of wells. This positioner cannot be used with 384-well plates that adhere to the SBS standards because the positioner does not align a plate according to the 4.5mm spacing grid of the standard plates.

See Figure 7-11 for illustrations of microplate well-center-to-well-center spacing.

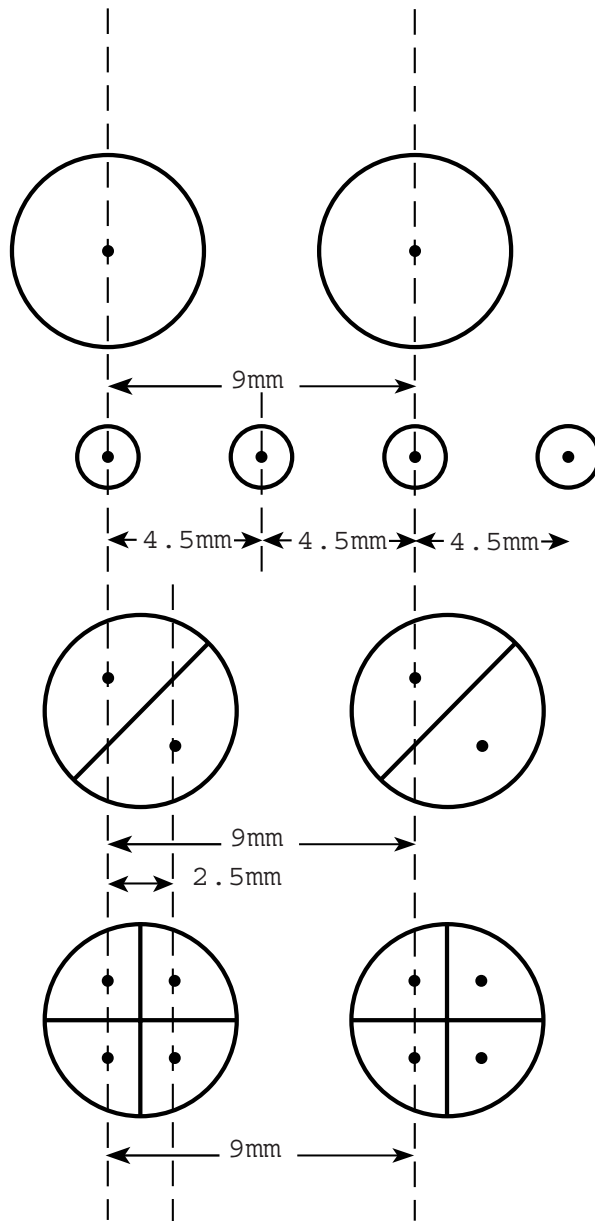


Figure 7-11: Well-Center-to-Well-Center Spacing for SBS Microplates and Apogent Discoveries' CyclePlate-192 and -384 ET Microplates

Using the Hydra-96 Plate Positioner for Standard 384-Well Plates

1. Place the positioner on the Hydra II 96 tray table.
2. Load the plate onto the positioner. (The CyclePlate-384DW ("discrete well," thin wall) microplate requires that Apogent Discoveries' removable adapter tray (Apogent Discoveries' Catalog No. 1029-41-2) be placed on the positioner first to secure the plate properly.)
3. Turn the thumbwheel on the front of the positioner to move it sequentially to each of four positions for dispensing or aspirating liquid to or from each of the four sets of 96 wells in the 384-well plate.

Using the Hydra-96 Plate Positioner for CyclePlate-384 and -192 Plates

The split-well positioner is used in much the same way as the standard positioner. You may find it more efficient to load the CyclePlate microplates onto the positioner with the positioner removed from the Hydra tray table.

Dispensing Onto the Sidewalls of Wells in CyclePlate-ET Plates With a Plate Positioner

See page 7-14 for information on using the Hydra-96 Plate Positioner for CyclePlate-384 and -192 (Apogent Discoveries' split-well, manually operated plate positioner) to dispense solutions onto the sidewalls of wells in CyclePlate-96, -192, or -384ET microplates.

Creating Membrane Arrays With the Vacuum Manifold and a Plate Positioner

You can use the Hydra-96 Plate Positioner for Standard 384 Well Plates with the vacuum manifold to create membrane arrays of up to 384 samples. See "Creating Dot Blots and Membrane Arrays With the Vacuum Manifold" on page 7-17 for more information about this use of the manifold and plate positioner for creating 384-sample membrane arrays.

Using the Hydra II 96 With R/B Syringes With Resin Suspensions

The Hydra II 96 system with R/B syringes was designed to allow multichannel dispensing of insoluble resins and beads into 96-well microplates or blocks. The syringe design for this Hydra II model incorporates a larger-bore needle than those of the Hydra II 96 and Hydra II 384 base-unit systems with standard stainless-steel and DuraFlex syringes. It also has a conical-shaped plunger tip and receptacle. These design features facilitate the passage of the suspensions, preventing the solid particles from collecting inside the barrels and clogging the syringes. When presented as homogenous suspensions, beads of up to 300 μ m in diameter (dry) have been drawn into the syringe barrels of the Hydra II 96 system with R/B syringes and dispensed uniformly into 96-well receiving plates and blocks.

General Guidelines for Using the Microdispenser With Resin Suspensions

Keep these two points in mind as you use the Hydra II 96 system with R/B syringes with resin suspensions:

- The most important factor in the accuracy of delivery is that the suspension be maintained in a homogeneous state so that each syringe in the array and each dispense will contain the same concentration of the bead or resin.
- Each resin has its own characteristic behavior in suspension. Some resins have been dispensed in concentrations of up to 5%, while

others can only be transferred when in concentrations of 2% or less. The principal difficulty for using some resins at the higher concentrations is that the particles begin to clump and aggregate, which can clog the syringes. Each new resin should be thoroughly tested by gradually increasing the percent concentration to verify the highest concentration that will allow trouble-free dispensing with the Hydra II 96 system with R/B syringes.

Note: If you have questions about types of beads and resins that can be used with the microdispenser, contact your equipment provider. See page 1-11 for contact information.



Examples of Types of Materials Used in Resin Suspensions on the Hydra II 96 System With R/B Syringes

Examples of the types of solid materials that have been successfully used with the Hydra II 96 system with R/B syringes and descriptions of its use follow.

Table 7-6: Examples of Materials Dispensed With the Hydra II 96 System With R/B Syringes

Suspension Material	Bead Diameter Before Swelling	Concentration Tested
Sephadex® G-50	=<160µm	2%
Tentagel S NH2 (in DCM/DMF)	300µm	2%
Yttrium Silicate (for SPA)	2 – 5µm	20mg/mL
PVT (for SPA)	5µm	20mg/mL
CPG	500 angstrom	0.25% (w/v)

- **Solid supports for chemistry**—The solid supports used in chemical syntheses can be transferred using the Hydra II 96 system with R/B syringes when they are prepared as an isobuoyant mixture. Typically, the amount of resin needed to give a 2% (w/v) solution is weighed out and mixed with the appropriate volume of 1:1 DCM/DMF (dichloromethane/dimethylformamide). Small volumes (1mL – 5mL) of DCM or DMF are added incrementally to cause the resin to rise or settle respectively within the solvent mix. Eventually the solvent mix is adjusted so that the resin neither rises nor settles. It is isobuoyant and is said to “float.” The suspension can then be transferred into a receiving plate or block. Rink resins, Wang resins, Tentagels, ArgoPore® and ArgoGel® (Argonaut Technologies), SPA (scintillation proximity assay, Amersham Pharmacia BioTech) beads, and others have been successfully transferred using this method with the Hydra II 96 system with R/B syringes.
- **SPA beads**—For this application, SPA (scintillation proximity assay, Amersham Pharmacia BioTech) beads are suspended in an

aqueous solution. They have a tendency to settle. This settling could present a problem if the beads were large or tended to form aggregates. But SPA beads are generally sized on the order of 5 μ m and remain in a uniform suspension long enough to be drawn into the syringes of the Hydra II 96 system with R/B syringes and subsequently dispensed. When SPA beads of yttrium silicate were tested in Apogent Discoveries' laboratories, it was found that even if the beads were allowed to settle within the syringe barrels, the Hydra II 96 system with R/B syringes was still able to dispense them with uniform results across the syringe array.

- **Sephadex beads**—Sephadex (Amersham Pharmacia Biotech) beads have been used in applications in high-throughput laboratories using 96-well filtration blocks to purify cycle-sequencing reaction products prior to loading onto the gel of an automated sequencer. (For more information about this application, see the article “Reduced Volume AmpliTaq DNA Polymerase FS Cycle Sequencing Reactions in the Robbins CyclePlate® 384” on the Hydra Technical Data page on the Apogent Discoveries website at <http://www.apogentdiscoveries.com/>). The Hydra II 96 system with R/B syringes is used to transfer the Sephadex slurry into the filtration plates. Despite the fact that the suspension settles quickly within the syringes, Sephadex G-50 beads of less than 160 μ m diameter can be drawn into the syringes uniformly and dispensed even after the beads have settled.
- **CPG beads**—CPG (controlled pore glass) beads have been used as the solid support in protocols designed for the MerMade DNA oligonucleotide synthesizer (available from BioAutomation). The diameter of these beads averages approximately 500 angstrom and does not clog the syringes despite the beads' near-immediate settling within the syringe barrel.

Using Viscous Solutions

In general, do not use the microdispenser to dispense viscous solutions. The microdispenser is designed primarily to dispense clear, aqueous solutions of low viscosity.

Hydra II microdispensers are available with syringe sizes ranging from 100 μ L up to 1mL. Apogent Discoveries studied the dispensing limits for several Hydra microdispenser models. Test results indicated that the larger bore syringe-and-needle combinations are capable of filling and dispensing the more viscous solutions. For example, the 1mL Hydra II 96 R/B syringe fitted with the widest needle available was able to fill and dispense glycerol solutions (glycerol in water) of up to 90% glycerol. It could also dispense methylcellulose/water solutions of up to 1000 centipoises (cps) viscosity. The tests also indicated that the 100 μ L syringes should be used with glycerol-in-water solutions of less than 50% glycerol for the Hydra II 384 and less than 60% glycerol for the Hydra II 96.

Data for the methylcellulose solutions showed that both the Hydra II 96 and the Hydra II 384 with 100 μ L syringes should be used with methylcellulose solutions of less than 26 cps viscosity.

Table 7-7: Pass/Fail Test Results for Filling Syringes to Full Volume With Solutions of Varying Percent Glycerol Composition

	Percent Glycerol						
	30	50	60	75	80	90	100
Hydra II 384 100 μ L	+	+	-				
Hydra II 96 100 μ L	+	+	+	-			
Hydra II 96 290 μ L	+	+	+	+	+	-	
Hydra R/B 1mL	+	+	+	+	+	+	-

Table 7-8: Pass/Fail Test Results for Filling Syringes to Full Volume With Methylcellulose Solutions of Varying Viscosity

	Viscosity (cps)						
	25	50	100	250	400	1000	1500
Hydra II 384 100 μ L	+	-					
Hydra II 96 100 μ L	+	-					
Hydra II 96 290 μ L	+	+	+	-			
Hydra II 96 with R/B 1mL	+	+	+	+	+	+	-

For more information about these tests, refer to the article “Dispensing Viscous Fluids with the Hydra Microdispenser” on the Hydra Technical Data page on the ApogentDiscoveries website at <http://www.apogentdiscoveries.com/>.

If you have any questions about using the microdispenser to dispense solutions with some limited viscosity, contact your equipment provider. See page 1-11 for contact information.

Ensuring Compatibility of Chemicals Used With the Hydra II Microdispenser

To protect your microdispenser from damage and to ensure the integrity of your results, you need to know which chemicals (acids, bases, organics, etc.) can be safely used with the microdispenser and how to use them.

The basic guideline for the use of corrosive or oxidizing agents with the microdispenser is not to allow such agents (chlorine bleach, strong acid, etc.) to be in continuous contact with the microdispenser. Such agents can be used routinely with the Hydra II microdispenser but may damage the case, the tray table/stage, and/or the glass syringes if left in contact with the instrument for extended periods of

time. If you need to use these reagents in a protocol, ensure that you clean the microdispenser of any residual corrosive agent as soon as you finish the procedure. **If you need to use any strong oxidizing or corrosive agent other than those listed in Table 7-9, contact your equipment provider.** See page 1-11 for contact information. They will give you guidance on how to use these agents in such a way as to protect the microdispenser and to ensure the integrity of your samples.

For details about using chemicals such as bleach in Wash protocols, see “Washing Syringes” on page 6-3.

Table 7-9 lists chemical reagents that have been used routinely in the Hydra II microdispensers.

Table 7-9: Chemical Reagents Used Routinely With Hydra II Systems

Acetic acid	Diisopropylamine	Methanol	Trifluoroacetic Acid
Acetone	Dimethylacetamide	Methylene Chloride	Trimethyl Orthoformate
Acetonitrile	Dimethyl Formamide	Methylene Chloride/ TFA, 1:1	Xylenes
Ammonia 27-30%	Dimethyl Sulfoxide	Pyridine	Toluene
Chloroform	Ethanol	Triethylamine	
Dichloroethane	Hexane	Toluene	

Preventing Solutions From Wicking Up the Sides of the Needles

If drops of solution are wicking up the sides of needles after a dispense, it is an indication that there is a buildup of residue on the needles and that the needles need to be cleaned. Preventing creep at the needle tip is of particular importance when attempting to effect a touch off during a dispense onto a dry surface. If there is any wicking it is possible that the sample will remain on the needle and will not be transferred to the dry surface. To solve the problem of drops wicking up the needles, follow the procedures given in “Cleaning Needles” on page 6-14.

Restoring Air Bubbles in Syringes

When syringes are filled to full volume you will notice a small air bubble fills the space between the syringe plunger tip and the solution. This air bubble represents the “dead volume” of the needle that is filled with air when the syringe is empty. Sometimes a portion of a solution will bypass the air bubble and fill the space normally occupied by the air bubble. After the air bubble has been replaced by a dispense solution, it is possible that some small amount of the solution may contaminate future dispense solutions.

To restore the air bubble, follow the procedure below.

Note: See page 6-5 for a glossary of wash-operation terminology.



1. Empty the syringes into a microplate or reservoir.
2. Wash the syringes with 3 wash cycles of dH₂O.
3. Wash the syringes with up to 9 wash cycles with Coulter® Clenz (Beckman Coulter cat. #s 8546929–31) diluted with water (50:50 detergent:water). Change the detergent solution at the end of each step.
4. Rinse the syringes with up to 9 wash cycles. Change the wash solution after every 3 cycles.
5. Wash the syringes with 3 wash cycles of ethanol.
6. With the syringes now empty, remove the ethanol reservoir. Press the **Wash** button to fill the syringes with air, and turn off the power by pressing the power switch on the rear of the microdispenser to **OFF** as the plungers reach full height.
7. Leave the plungers in the fully retracted position and allow the syringes to dry (overnight if necessary).
8. On the next day, turn on the microdispenser and wash the syringes with 3 wash cycles of dH₂O.

