

# Vacuum Manifold for Oligo Cleavage



5437 Central Ave, Suite 5 • Newark, CA 94560 • 510.795.1142 • Fax 510.795.1149 • www.Biolytic.com

## Operators Guide, Vacuum Manifold For Cleavage of Plate Oligos

This document will instruct you on the proper use of the Vacuum Manifold designed for cleaving oligos that have been synthesized in a 96 well plate or were transferred to a 96 well plate after synthesis.

### ***Theory of operation:***

This device is designed to allow for easy cleavage of synthesized oligos from the support. Cleaved oligos will be deposited in a deep well plate or in a rack of tubes depending on what the user prefers. An external (User Provided) vacuum source is used to move the cleavage reagent through the columns or wells containing the solid support bound Oligos. As the Cleavage reagent moves through the solid support, the Cleavage reagent will react with the linker that is attaching the Oligos to the solid support thus cleaving the Oligo from the solid support.

The Cleavage Reagent will now contain the cleaved Oligos. The Cleavage reagent is captured in wells of a deep well plate or in the tubes that are placed in the bottom of the vacuum manifold under the rack containing the synthesis columns.

One can choose to use either a deep well plate or a rack of tubes to capture the cleaved Oligos.

There are many different deep well plates available in the market. Some have round wells, some have square wells, some are deeper than others.

To accommodate a wide variety of deep well plates and racks of tubes, we supply a set of shims. The shims are placed in the bottom of the Vacuum Manifold first then the deep well plate or rack of tubes is placed on the top of the shims.

The purpose of the shims is to move the deep well plate or rack of tubes up so that the tips of the synthesis columns protrude into the open tops of the deep well plate or rack of tubes.

Figure 1, shows the Vacuum Manifold with some different size racks. In this figure the manifold is disassembled and ready to load for cleaving, The blue rack is a rack of tubes that the oligos can be cleaved into. A rack of tubes such as this or deep well plate can be used to cleave the oligos into.

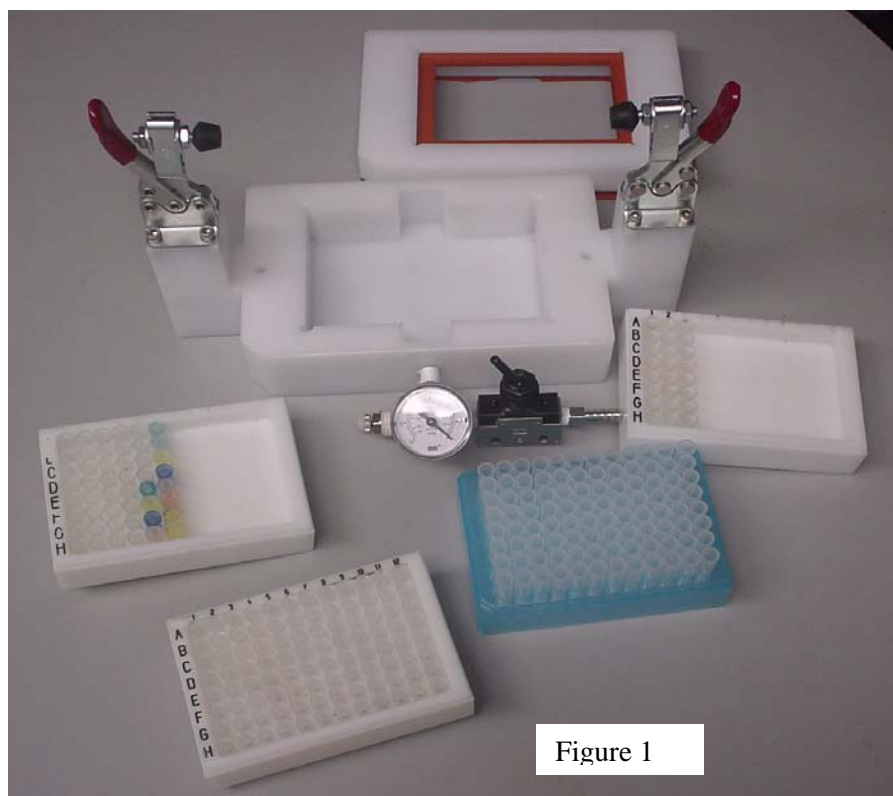


Figure 1



## ***Procedure for Cleavage:***

The Vacuum Manifold requires that you supply a vacuum source. The idea is to cleave the oligo from the support into a deep well plate.

It is recommended that this device be used inside of a properly vented fume hood as most cleavage reagents are volatile and odorous.

1. Place the Deep well plate into the bottom of this device, see figure 2 & 3.
  - a. Use shims under the plate to make it come up high enough so that the column tips from the synthesis plate protrude down into the wells of the deep well plate. We supply several shims with the system to use for this purpose. If the shims that we provided are not correct for your plate, you can use anything that will fit as a shim.
2. Place the top on the device, see figure 4
3. Place a rack of oligos to be cleaved on top of the device, see figure 5
4. Clamp the rack and the device together to make a seal, see figure 6, 7, 8
5. Pipette the cleavage reagent into the wells in the top rack using a multi channel pipette supplied by the user.
  - a. Note: If you are using the CTGen Frits, it is recommended that a small amount (50ul) of Acetonitrile be used to wet the frits before applying the cleavage reagent. This wetting of the frits will allow the frits to absorb the cleavage reagent easier.
6. Apply Vacuum to draw the cleavage reagent down into the support. Do not apply too much vacuum as the cleavage reagent will go immediately into the deep well plate if too much vacuum is applied.
7. Place a cover over the top of the rack to minimize evaporation. See figure
8. Apply Vacuum again after 10 minutes.
9. Repeat steps 5 thru 8 (perform 5 thru 8 a total of 4 times).
10. For the final step, turn the vacuum on longer and stronger to make sure that all of the cleavage reagent is drawn through the synthesis media and into the deep well plate.

Prior to removing the synthesis rack / plate from the top, use a plastic mallet to tap the top of the synthesis plate / rack to knock any drops off the bottoms of the synthesis columns that have not fallen off. This is very important. You do not want to have a drop fall into the wrong well and contaminate it.

**Note:** To use this device requires the following items which are not included with the Vacuum Manifold:

- A vacuum source such as a simple dry air vacuum pump.
- An 8 channel pipette 0 – 200 ul.
- Cleavage Reagent
- A fume Hood
- Deep Well Plate or rack of tubes.
- Racks to hold the synthesis columns



Figure 2 shows the blue rack of tubes loaded in the bottom of the vacuum manifold. These are usually called Cluster Tubes. If you ultimately need the oligos separated into individual tubes, cleaving into the Cluster Tubes in a rack as shown is a good way to go. Alternately you can cleave into a Deep Well Plate as shown in Figure 3. In either case, the cluster tubes or the Deep Well Plate should be made of Polypropylene.

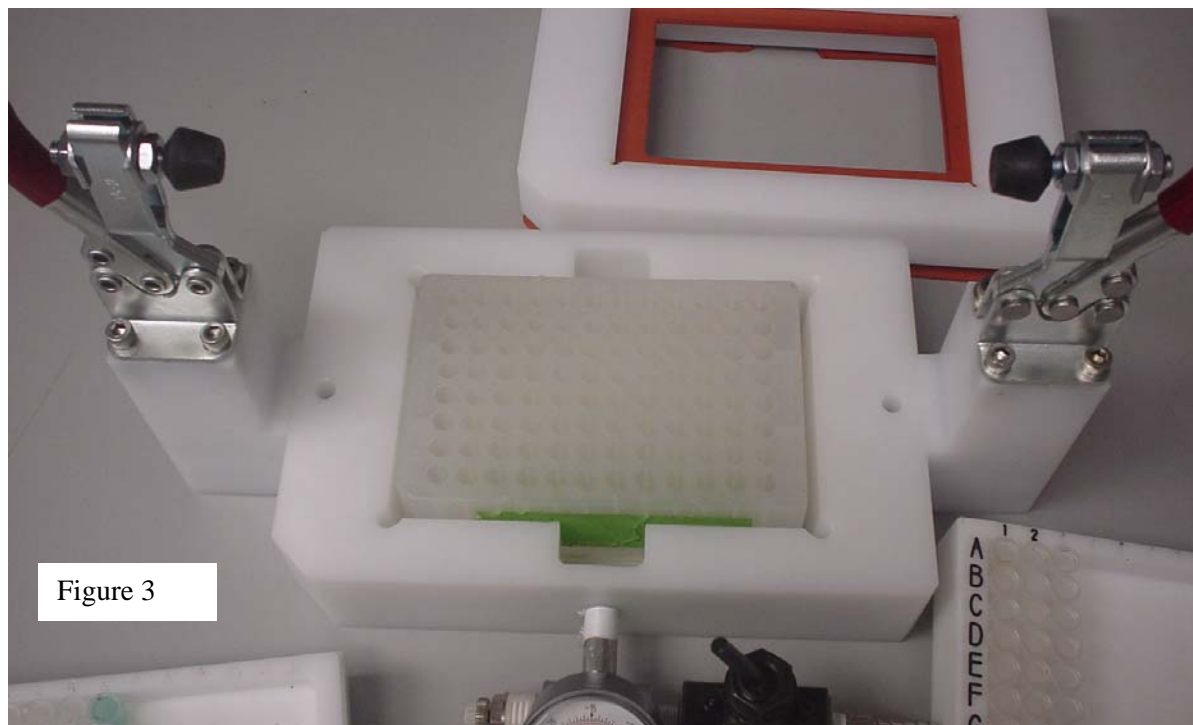
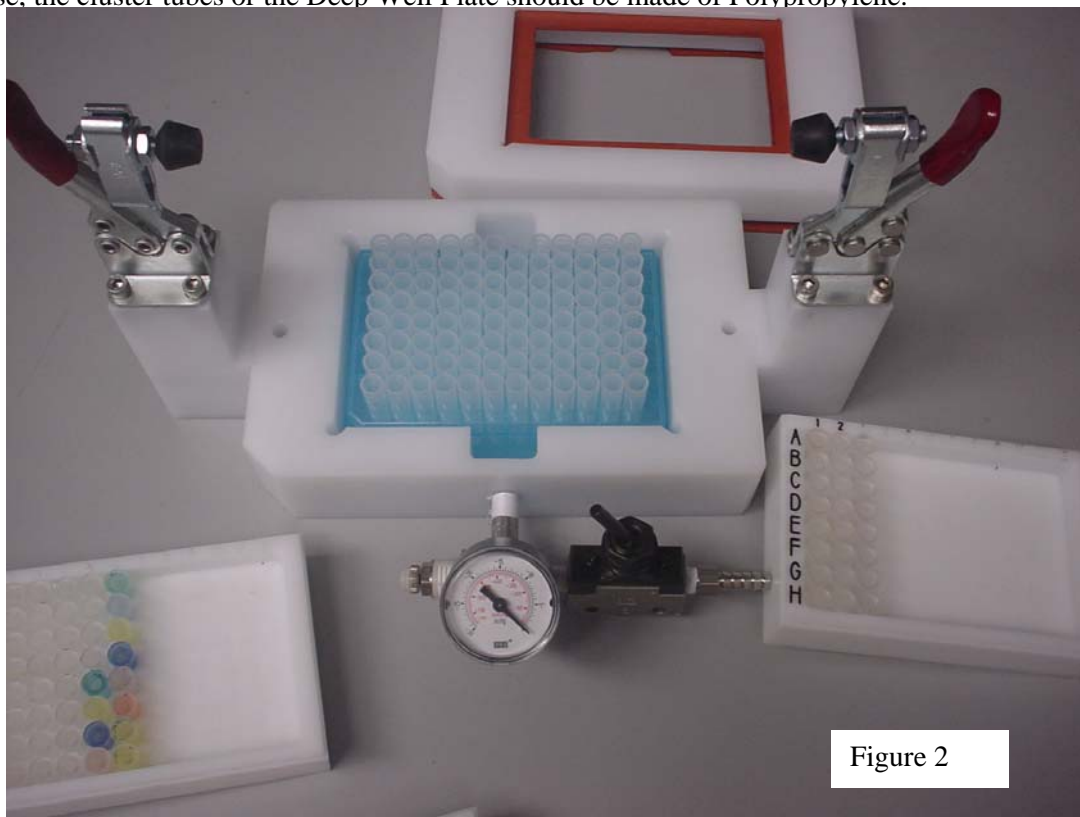




Figure 3 shows a rack of 96 Cluster Tubes loaded into the vacuum manifold.

Figure 4 shows the top placed on the vacuum manifold with the rack of tubes loaded in the bottom of the vacuum manifold. Figure 5 shows the plate of oligos on top of the vacuum manifold ready to cleave.

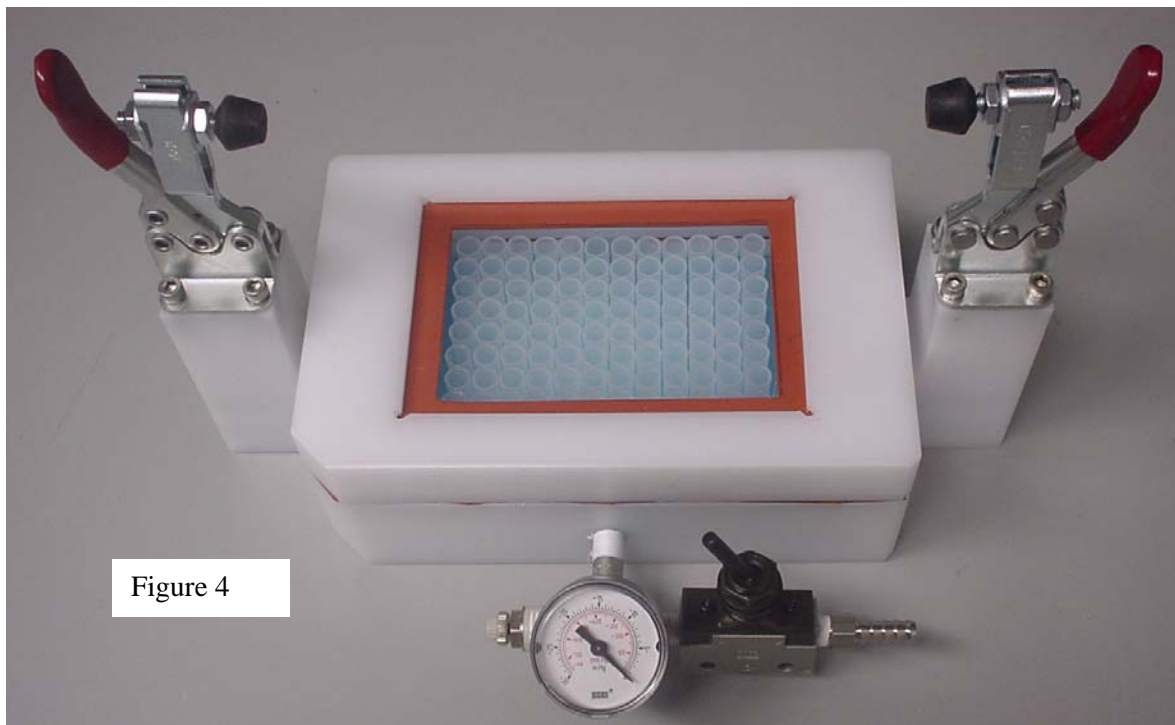


Figure 4

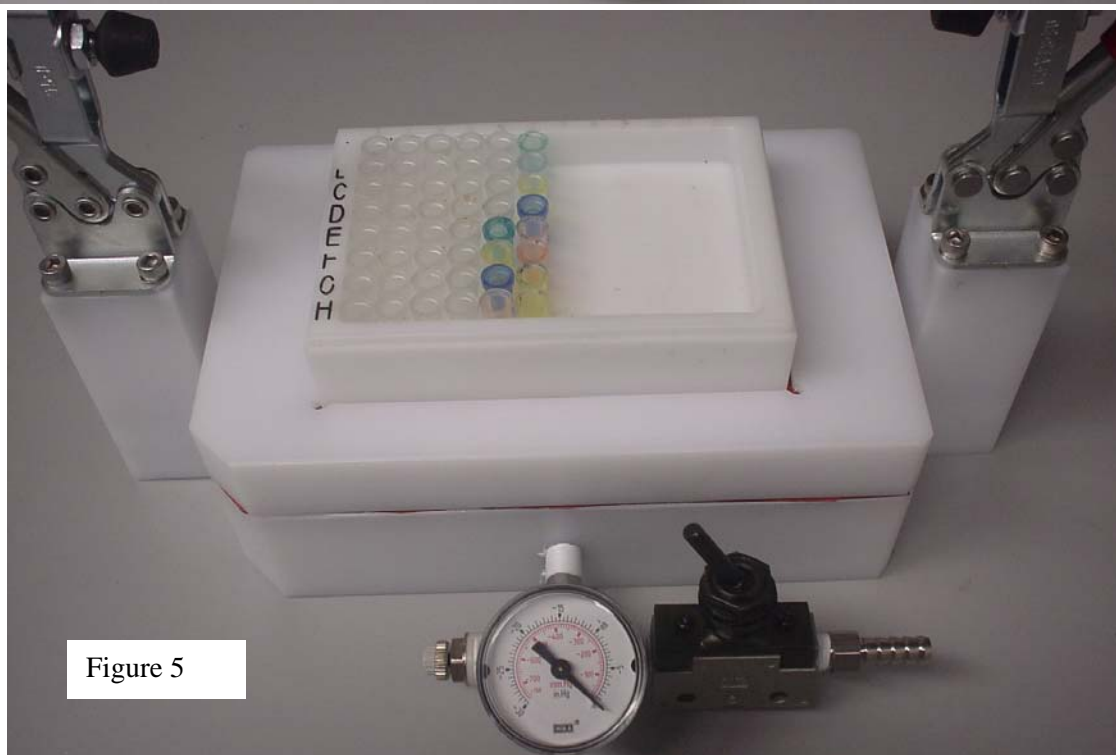


Figure 5



The Vacuum Manifold shown with the latches closed. The latches maintain a tight seal to the vacuum can be applied and maintained. Just press the red handles down to latch the plate and the vacuum manifold together.

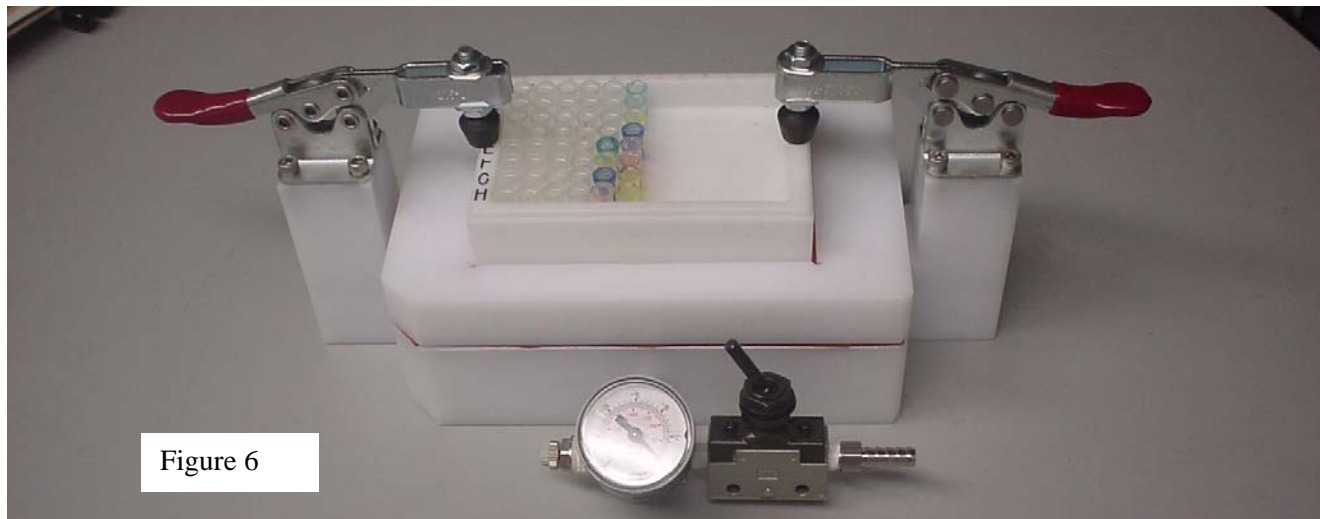


Figure 6



Figure 7

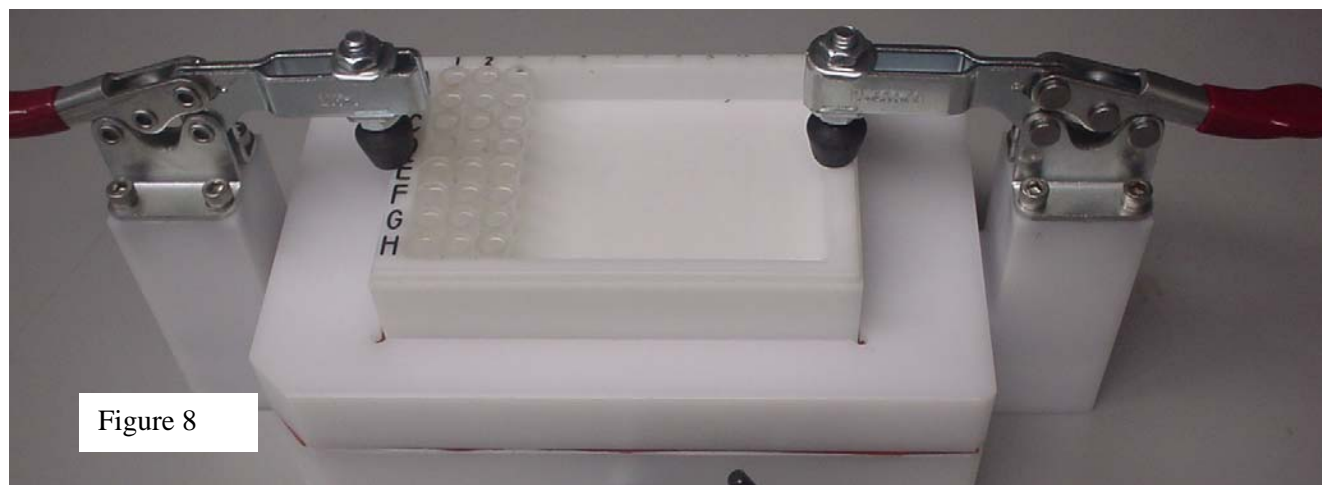
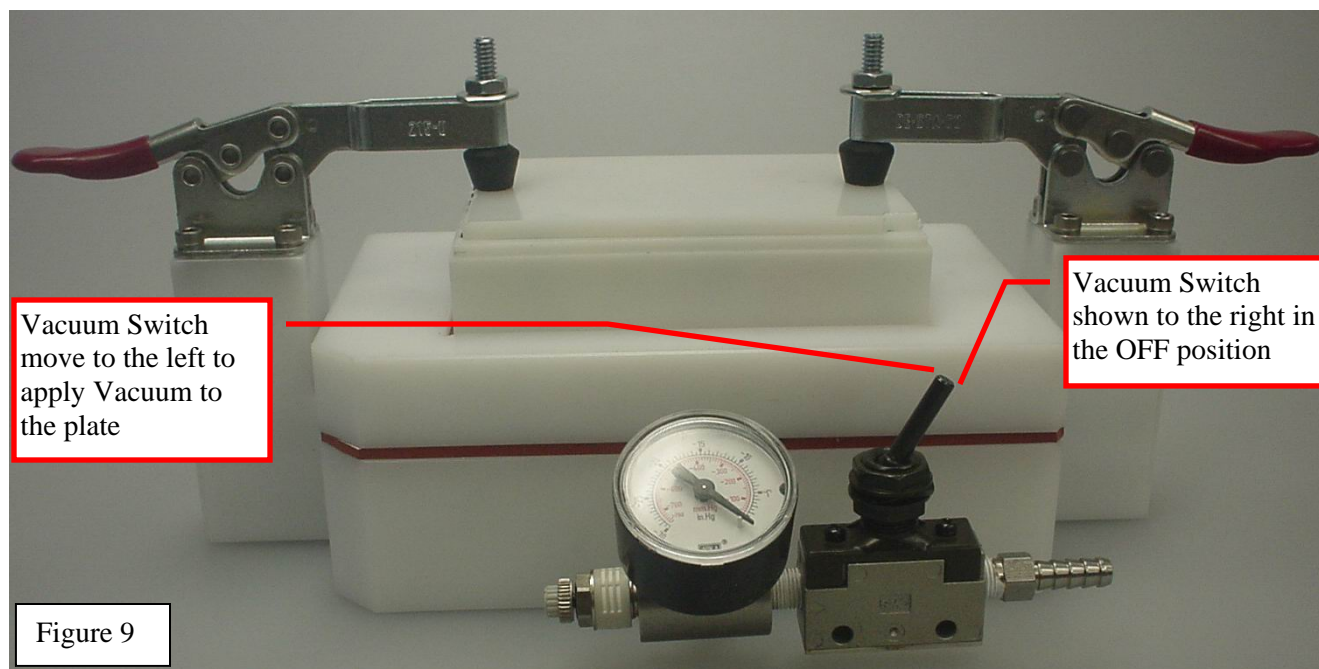


Figure 8



### ***Post Cleavage:***

When cleavage is complete, the oligo will be in the Deep Well Plate / Cluster Tubes suspended in about 800 to 1000 ul of cleavage reagent.

The next step is to deprotect the cleaved oligos.

1. Place a seal on top of the Deep Well Plate / Cluster Tubes. This seal can be a flat piece of Silicon that is about 1/8 in thick.
2. Place the Deep Well Plate / Cluster Tubes into a clamp designed to clamp the top tightly against the top of the wells to seal them and keep the Cleavage Reagent from escaping.
3. Place the clamped plate into an oven pre-heated to 75 degrees C.
4. After 2.0 to 4.0 hours, remove the clamped plate from the oven.
5. Place the hot, clamped plate in the freezer to cool it.
6. Make certain that the plate is cold before unclamping it.
7. Dry the Cleavage Reagent using a hot air dryer or a speed vac.
8. Re-suspend the oligos in DI Water and read the OD as desired.



The following pictures show some other examples of deep well plates with square holes, round holes and sealing covers.

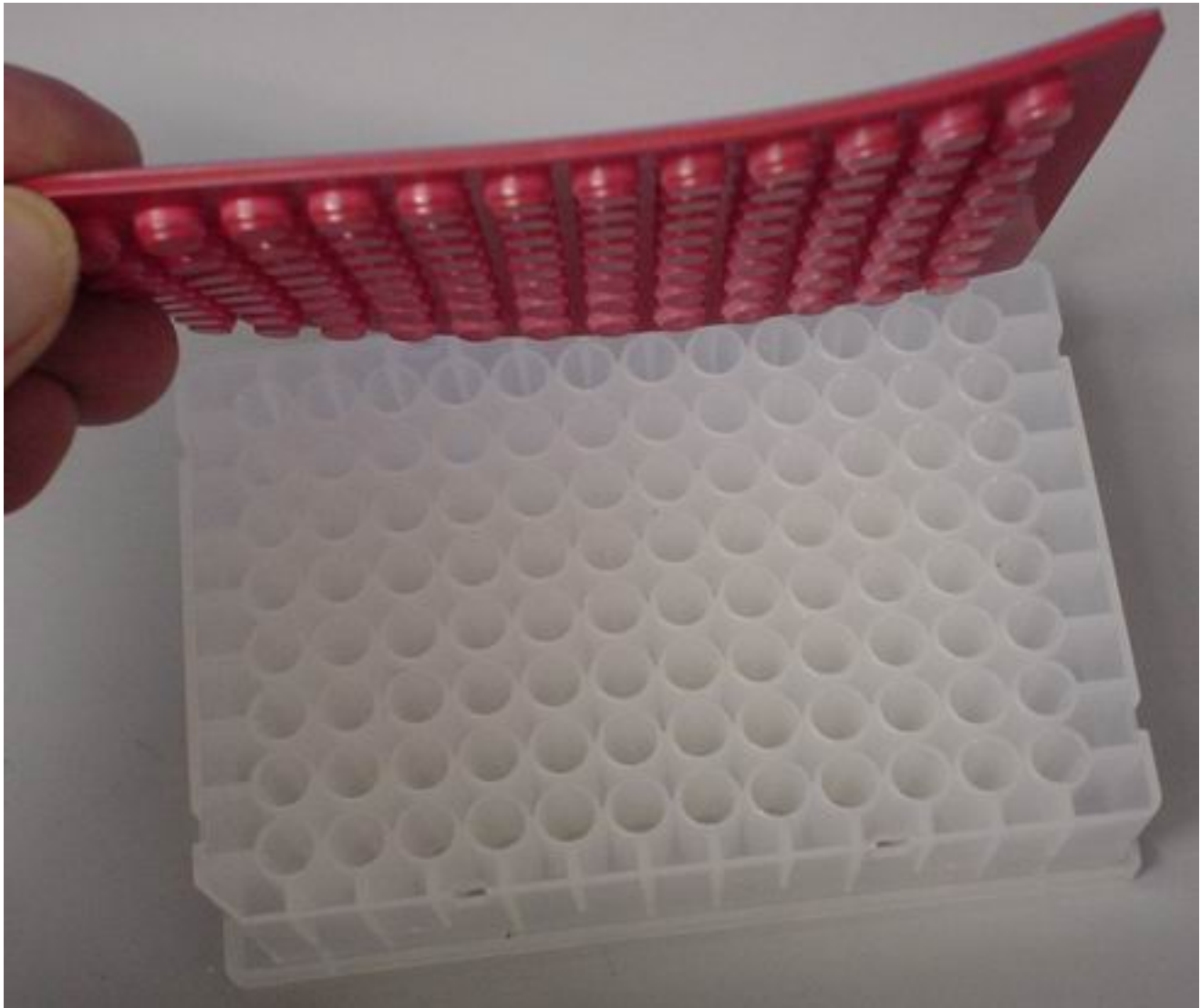
Biolytic Lab Performance, Inc. sells the square and round hole covers. Biolytic Lab Performance, Inc. does not sell the deep well plates or cluster tubes. These are available from most plate suppliers.

### **Polypropylene Deep Well Plate with round holes**





**Polypropylene Deep Well Plate with round holes and shown with a cover that has protrusions that will seal the wells.**





## **Polypropylene Deep Well Plate with square holes**





**Polypropylene Deep Well Plate with square holes and shown with a cover that has protrusions that will seal the wells.**

