MicroELISA Prototype

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Preface

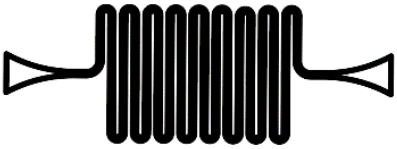
I had previously worked as a lab assistant at the Hughes Laboratory at the University of California, Irvine. My duties revolved around manufacturing microfluidic devices to assist in the lab's research.

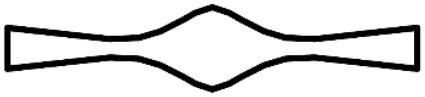
In the summer following graduation, I decided to volunteer at the lab and engaged in an independent research project.

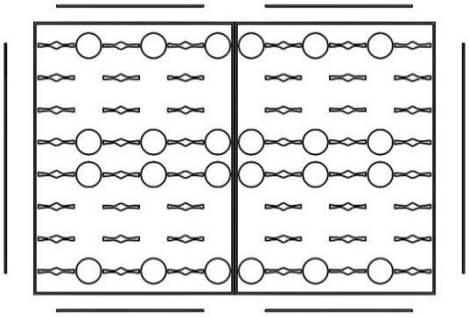
In 2016, from June until the start of September, I worked independently at the Hughes Laboratory, under the supervision of my advisor, Hugh Bender, towards designing a microfluidic device that was compatible with the ELISA (enzyme-linked immunosorbent assay) technique.

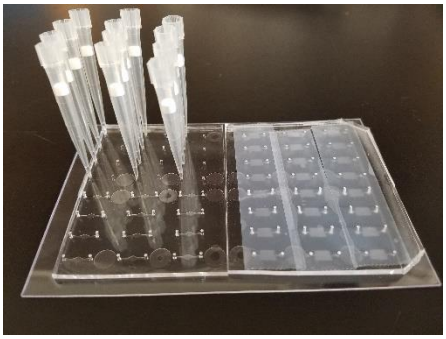
This portfolio documents my endeavors during this period.

Overview









Motivation

An ELISA (enzyme-linked immunosorbent assay) is a well-established technique to measure analytes; however, several disadvantages of the technique include reagent costs and tedious pipetting.

In this regard, would it be possible to leverage and adapt the Hughes Laboratory's microfluidic devices for use with ELISA? Specifically, the use of microfluidics would decrease the amount of required reagent and, thereby, both the cost and pipetting required.

Benefits

The device poses not only as a reduction in costs and pipetting, but also familiarity with known technology (microfluidics) but applied in a new application (ELISA). The benefits above stand to ultimately facilitate the further adoption of ELISA into the workflow of the Hughes Laboratory.

Design Goals

I established the following design guidelines, each followed by my reasoning:

1. Adapt the design of the Hughes Laboratory's current microfluidic devices.

   A familiar design reduces the time required by researchers to learn how to fabricate the new device.

2. Minimize the costs needed to fabricate one device.

   The device must optimize the limited budget I had available, a maximum of $200 for any additional materials not already found within the Hughes Laboratory.

3. The device must be analogous to a standard 96-well plate used with ELISA.

   The device must be compatible with the microscopy equipment currently used within the Hughes Laboratory; new equipment would increase costs and violate the 2nd guideline.

4. The device must be as translucent as possible to maximize the accuracy of absorbency measurements.

   ELISA is a technique that depends on microscopy; therefore, opaque materials would only sabotage the purpose of the micro-ELISA device.

5. Minimize the time required to fabricate the device to within 1-2 days.

   Faster fabrication facilitates maximizing my allotted time on the project: 3 months.

Design Results

1. The device combines the PDMS construction of the lab's current devices with newly designed channels and a polystyrene base sheet.

2. Aside from already available lab materials, the device requires polystyrene sheets and tools to cut the sheets; both are cheap and easily found at arts and crafts stores.

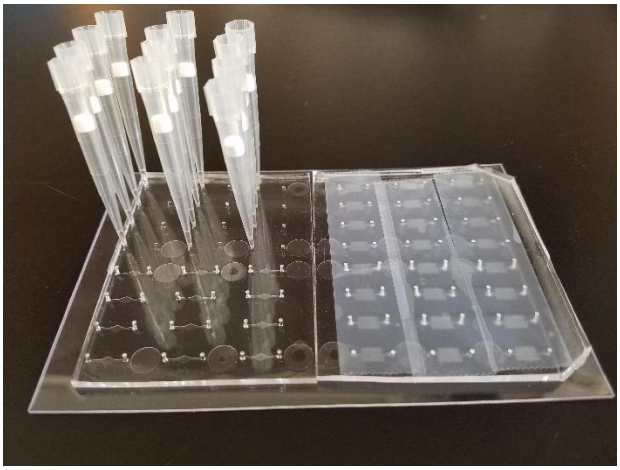
3. The polystyrene sheet is cut to the dimensions of a 96-well plate.

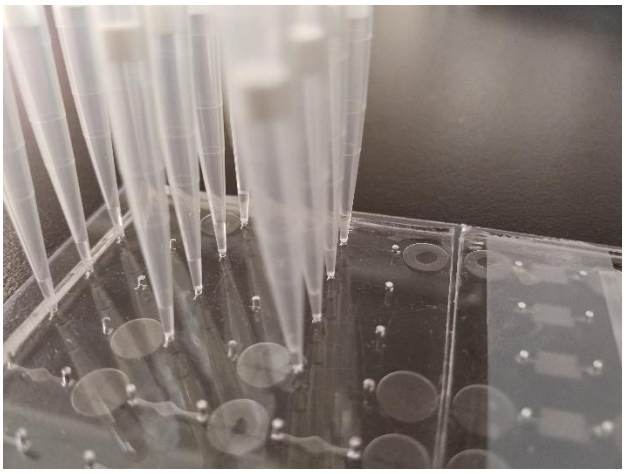
4. The additional polystyrene base sheet and the rest of the device's construction (PDMS) is both translucent.

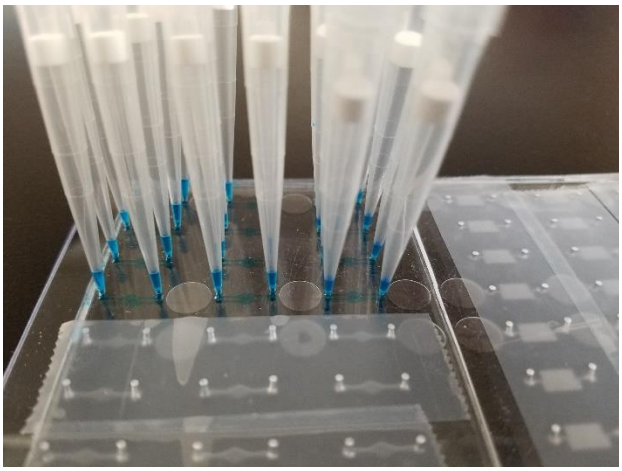
5. The device can be constructed within 2 hours and then prepared overnight. Total fabrication time is 24 hours.

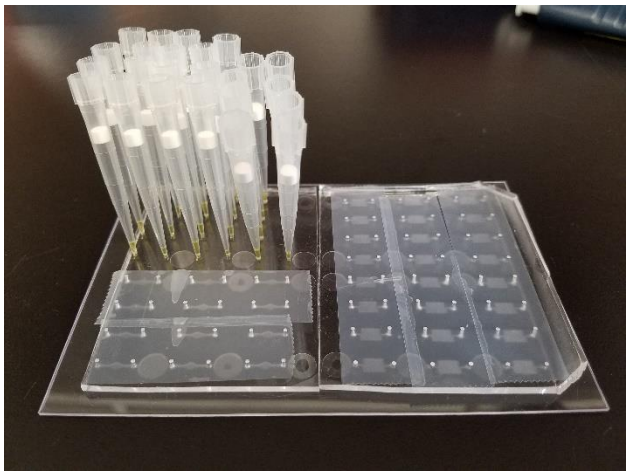
6. **The straight channels seem to be more effective than the serpentine design.**

Device Images

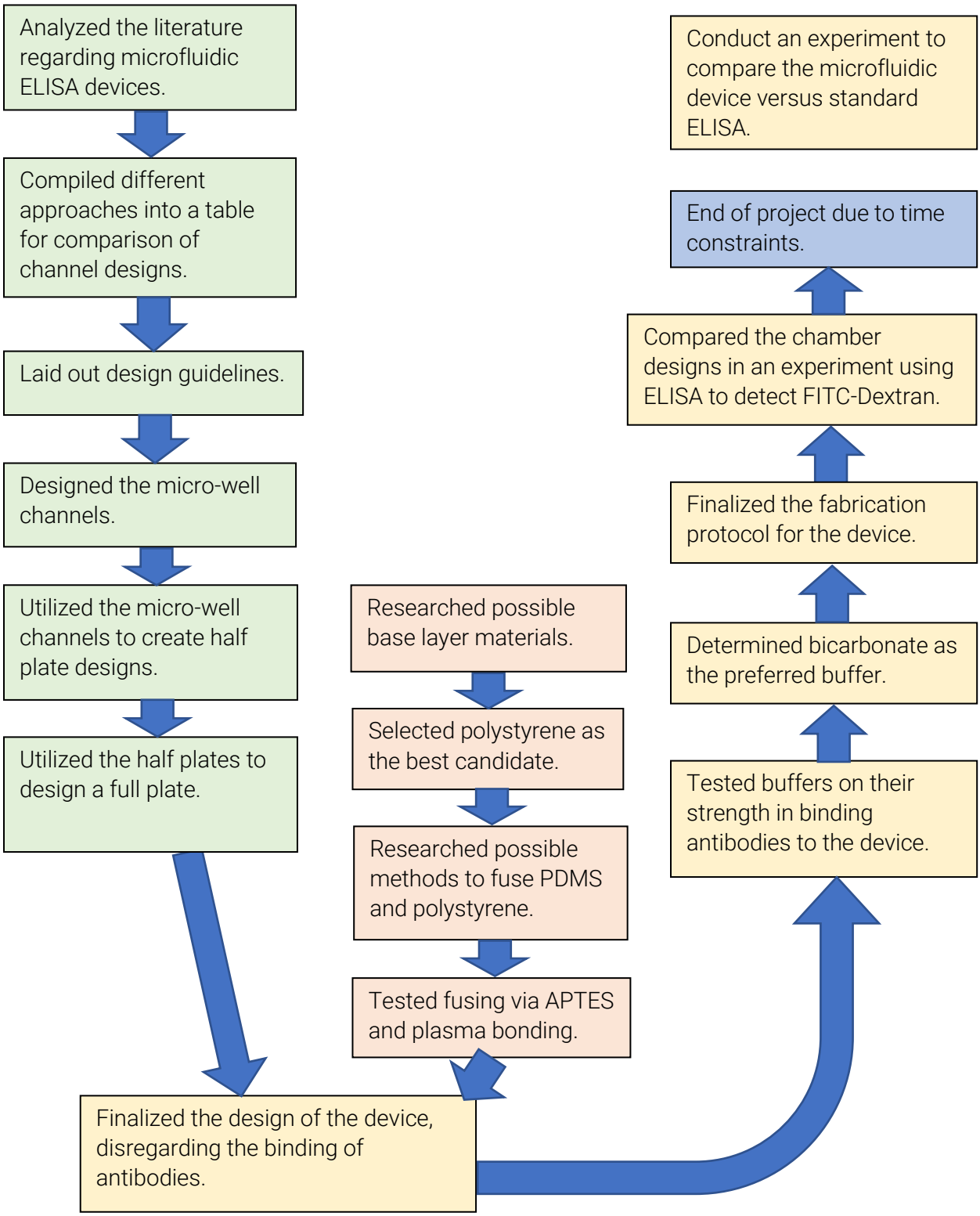








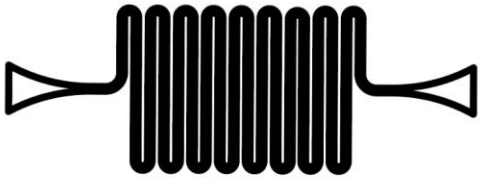
Project Progression



Design of Micro-Well Channels

Several micro-well channels were prototyped within SolidWorks and AutoCAD.

1. **Serpentine**



2. **Straight**

Rationale

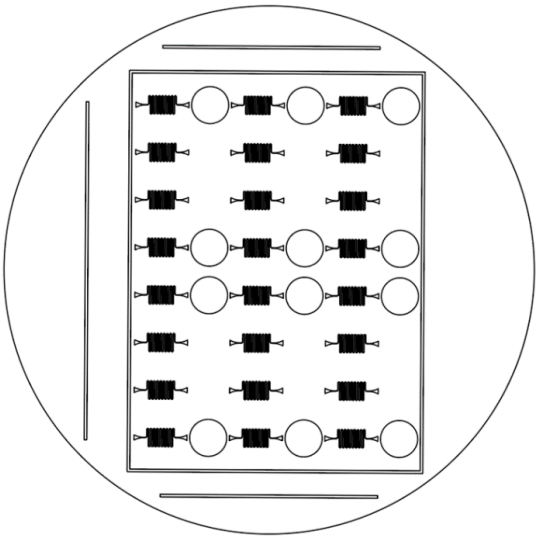
The straight channel was chosen as an excellent starting point, a generic baseline. Conversely, I designed the serpentine channels as an antithesis to see how narrower chambers and an increase in total surface area affect the baseline's behavior.

Design of the Half Plate (Half of 96-Well Plate)

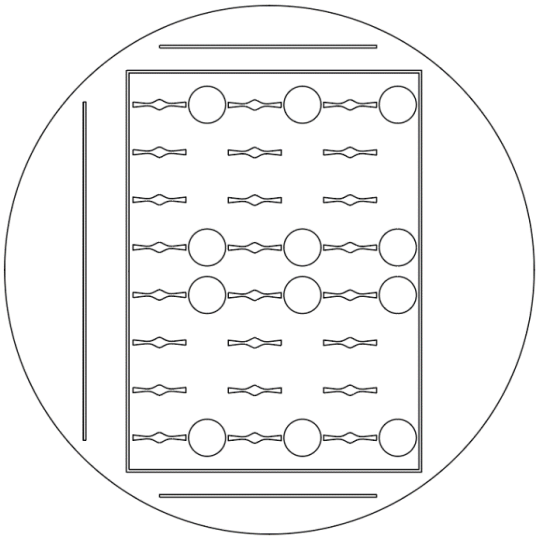
The designs for half of the 96-Well Plates were intended to fit within a standard petri dish.

Additionally, I added guiding lines to 3 sides of the design; because each half of the device is not symmetrical, the orientation of the halves is critical for correct assembly of the complete device. The absence of guiding lines indicates where the two halves should be joined.

1. **Serpentine**

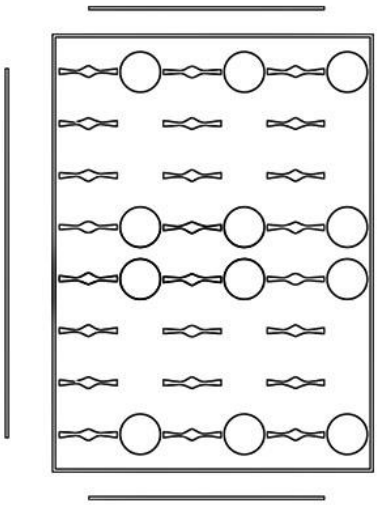


2. **Straight**

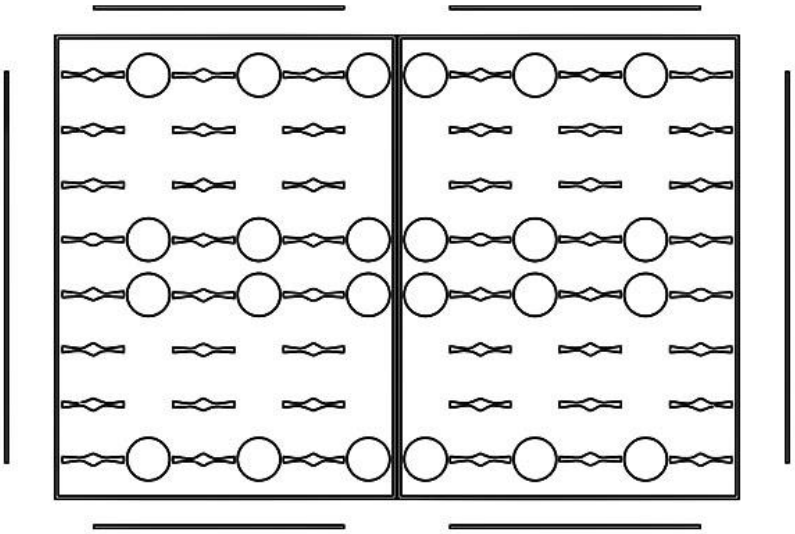


Design of the Full Plate (96-Well Plate)

Plastic molds were created from each of the half plate designs. PDMS can then be poured into the mold to construct half of the device.



By employing two of the half plate designs, the complete top-layer of the device (analogous to a standard 96-well plate typically used in ELISA) can be constructed.



The circles found in the designs above serve as landmarks. Each of them exactly corresponds to a standard well found on a 96-well plate. Therefore, the landmarks ensure that the polystyrene base layer can be correctly positioned and fused to the device.

Fabrication

Materials

1. **Plaskolite Clear 0.40”x9”x12” Sheet**
   MisterArt, Item #: 70428, Manufacturer #: AX70428

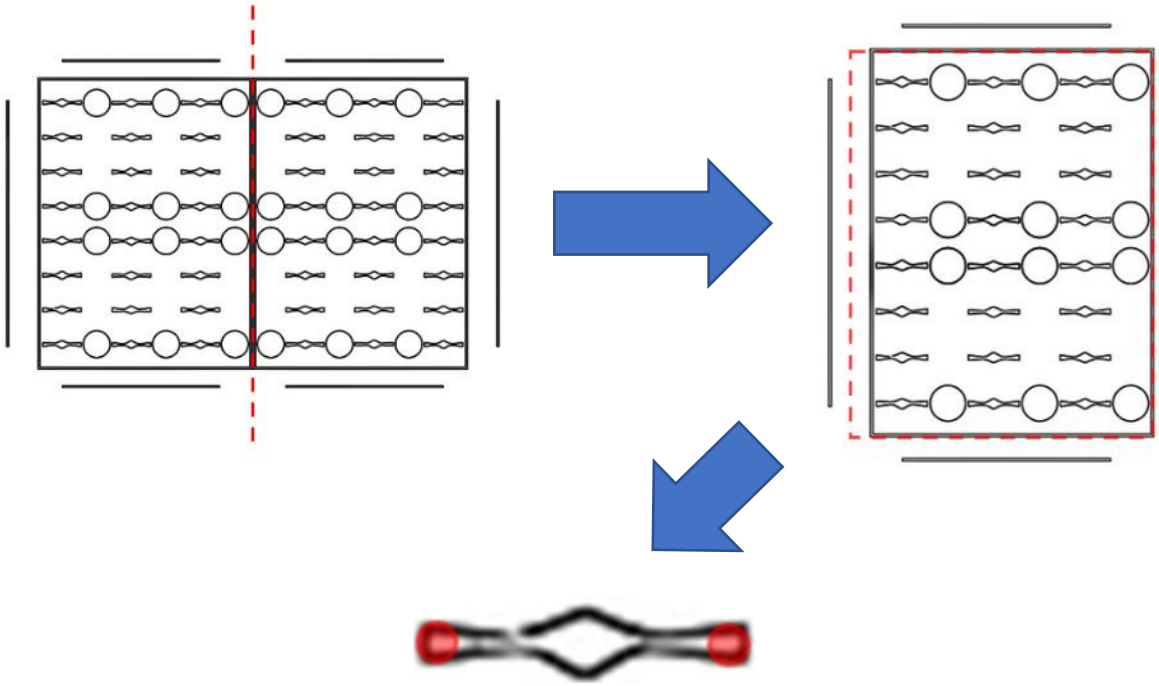
2. **Plaskolite Cutting Tool for Plastic Sheets**
   The Home Depot, Model: 1999999A, Internet #: 100542314, Store SKU #: 116491

3. **APTES**
   Sigma-Aldrich, SKU #: 440140

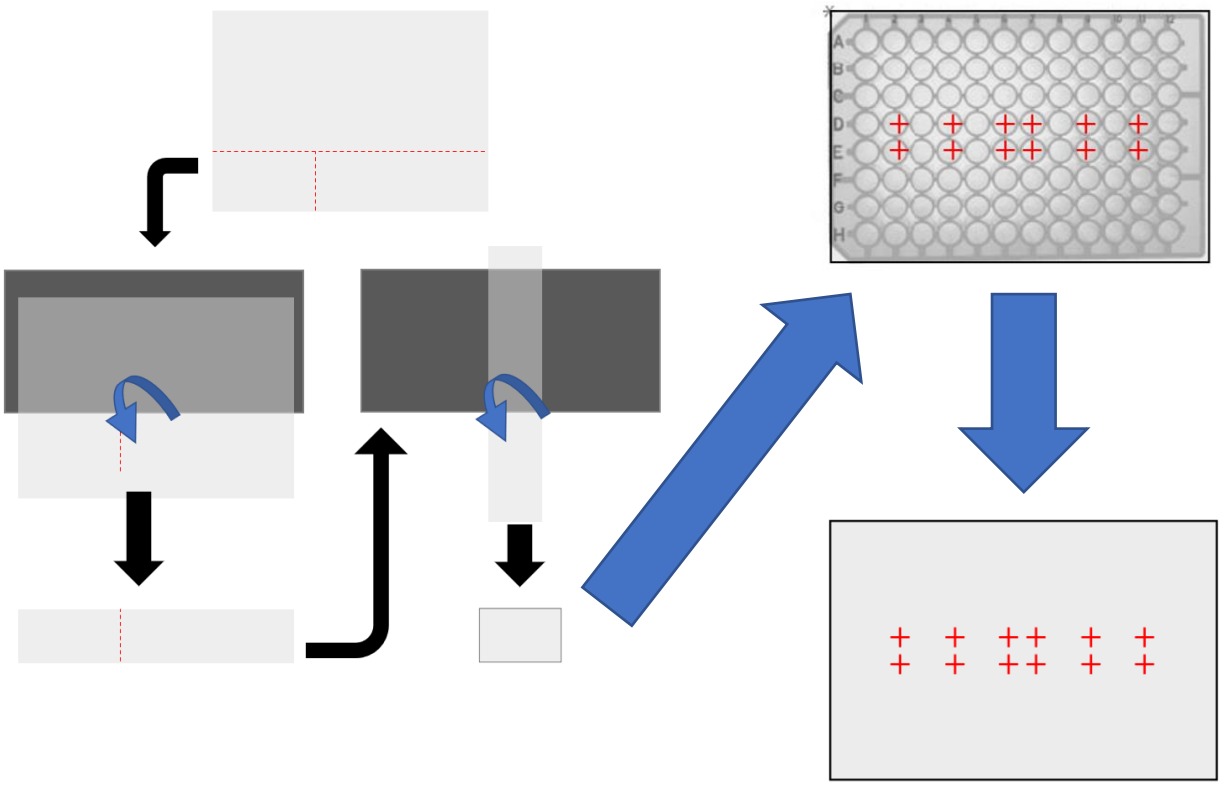
4. **PDMS: SYLGARD 184**
   Sigma-Aldrich, SKU #: 761036

Overview

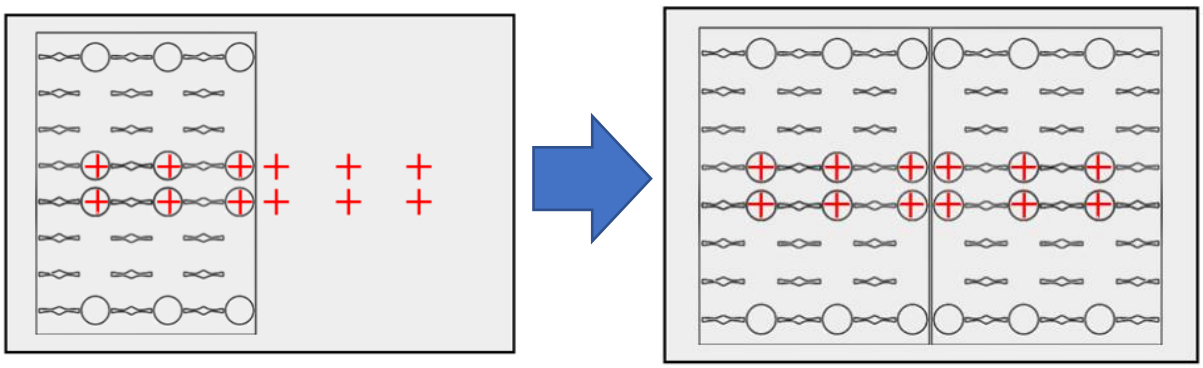
1. Prepare PDMS halves.



2. Prepare polystyrene base layer.

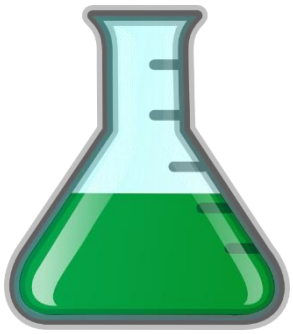


3. Form device by fusing PDMS halves to polystyrene base layer.

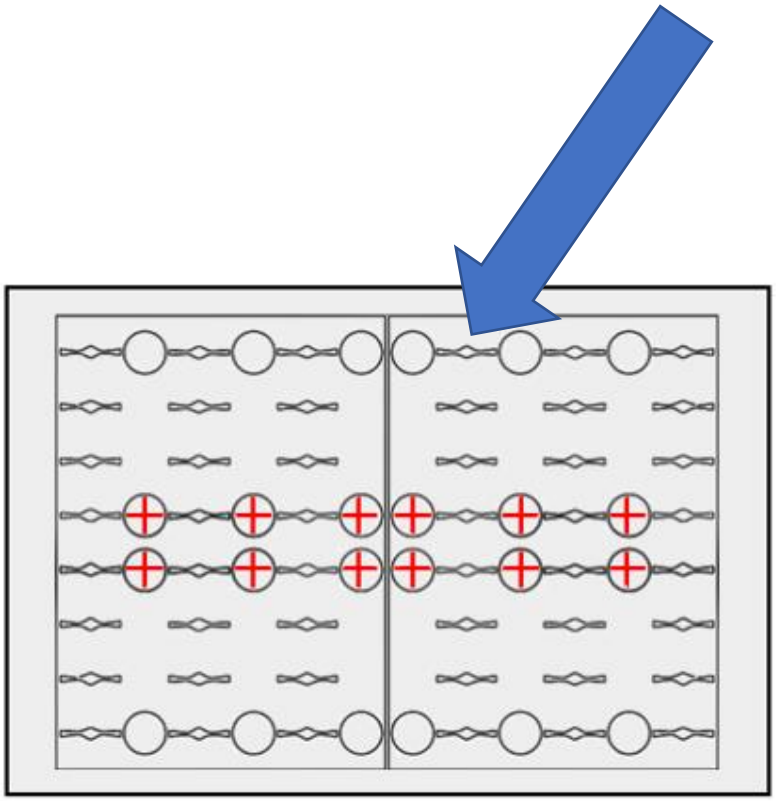


4. Bind desired primary antibodies to the device.

Primary Antibody

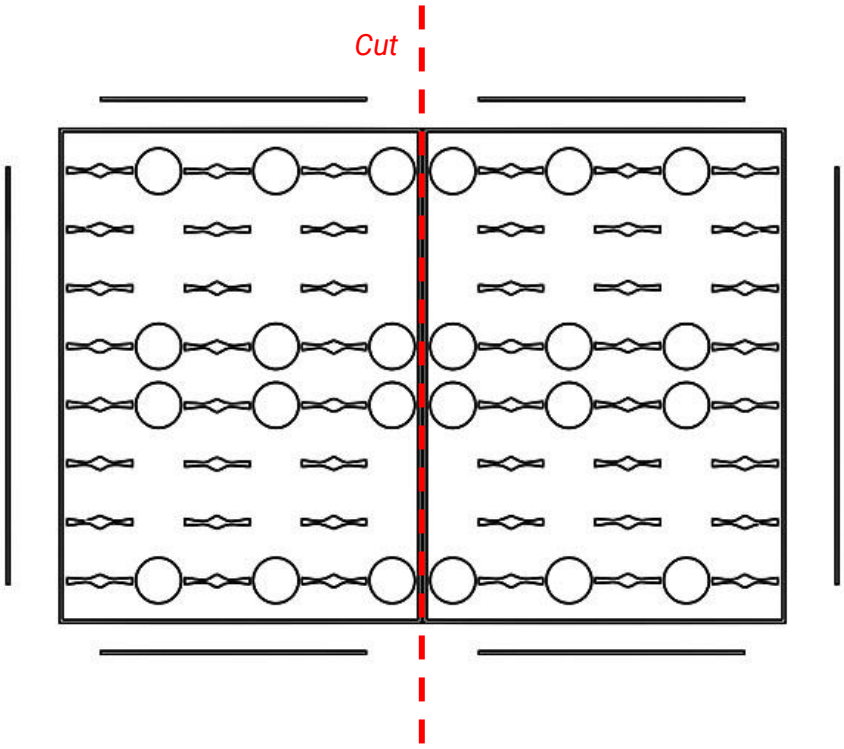


Bicarbonate Buffer

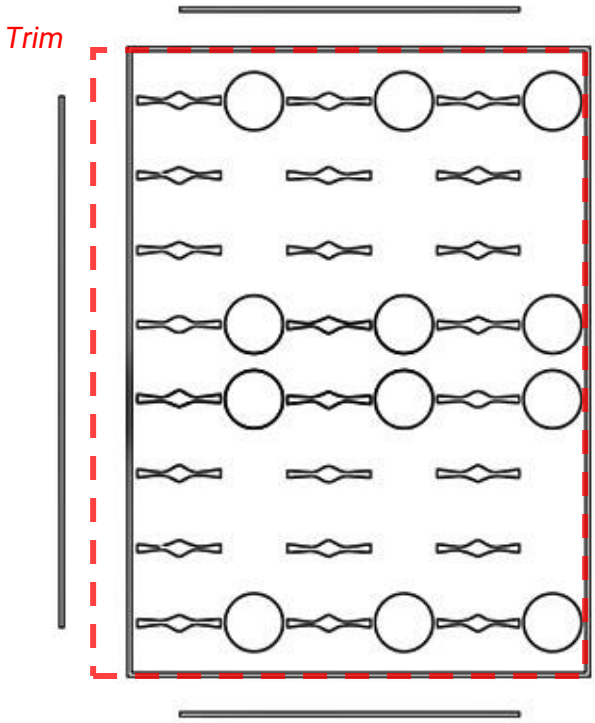


I. PDMS Preparation

1. Remove PDMS plastic mold.
2. Cut PDMS into two halves as shown below.



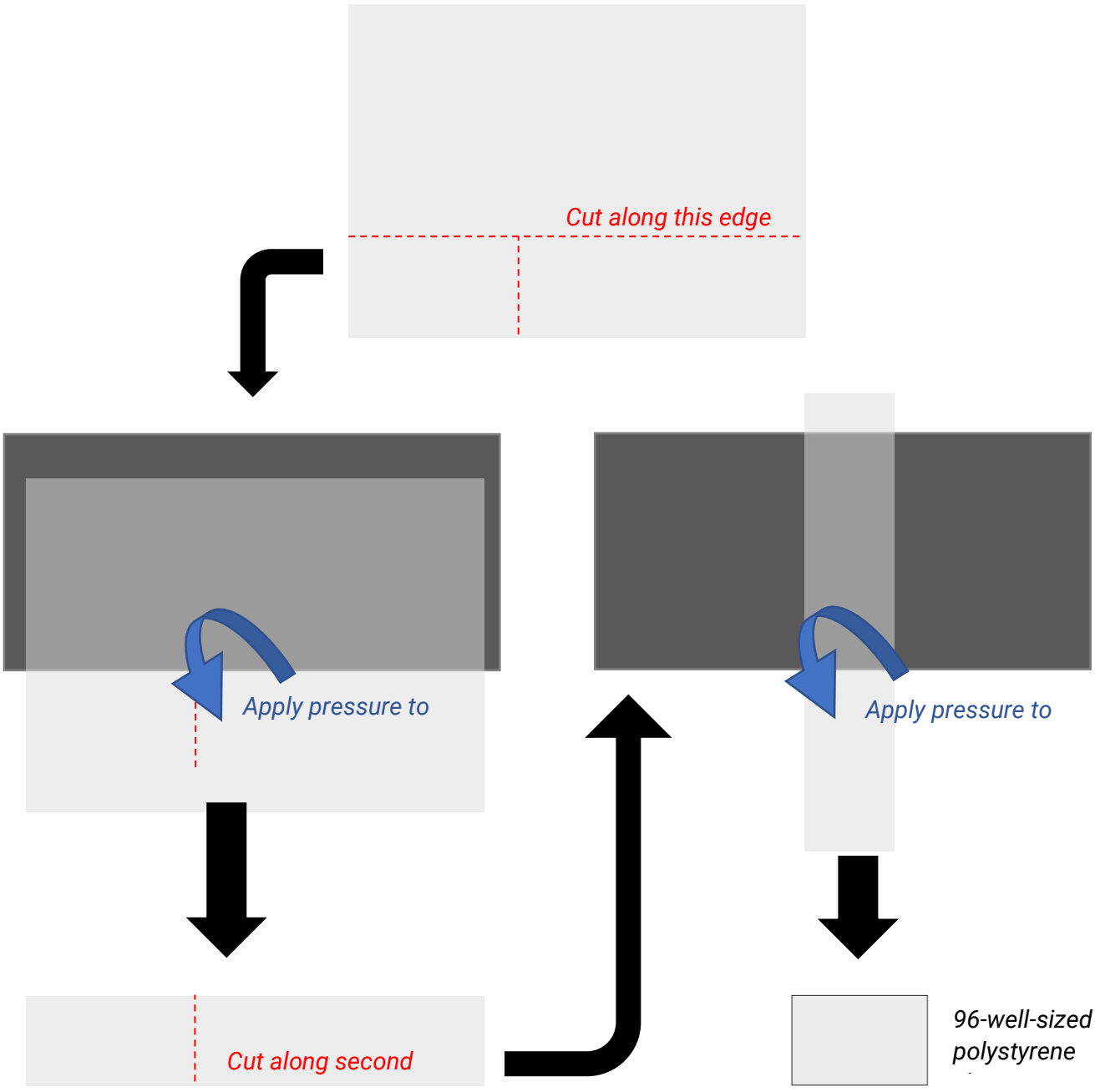
3. Trim edges of each half as shown below.



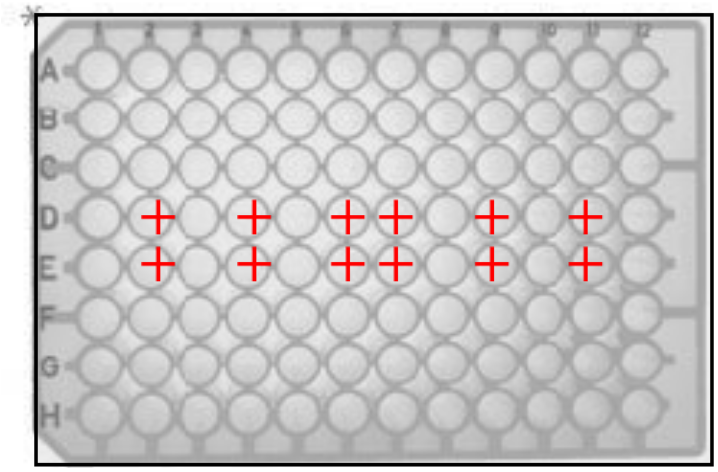
4. For each micro-well, use a biopsy punch to create loading-pores as shown below. Store PDMS halve in a clean location until ready to bond PDMS and polystyrene. Use tape to cover micro-wells from dust and other particulates.

II. Polystyrene Preparation

1. Use a straightedge and 96-well plate to trace out the amount of polystyrene required (approximately 128 mm x 85.5 mm).
2. Cut the polystyrene sheet by using the plastic cutting tool and straightedge. Usually, 3-4 cuts using moderate force along the traced lines will be sufficient.
3. Hold the polystyrene sheet off the edge of a flat surface.
4. Apply force to the cut region to snap the desired sheet size off.
   For Steps 1-4, refer to the flowchart below.



5. Place the appropriately sized sheet on top of a 96-well plate and draw well guides in the locations depicted below using an alcohol-resistant Sharpie.



III. Polystyrene Plasma Pre-Treatment

1. Put polystyrene sheets in the middle of the plasma chamber.
2. Make sure the needle valve is closed completely.
3. Close the door and hold it, then turn ON the pump.
4. Wait for a few seconds and then check that the door cannot open.
5. Check the pressure gauge, the needle should start going down.
6. Allow the machine to pump down and wait until pressure reaches 140 to 160 mTorr. This step ensures that all particles are removed from within the plasma chamber. This process should not take longer than 2 minutes.
7. Slowly open the needle valve and maintain pressure between 200 to 300 mTorr.
8. Turn ON the plasma, adjust needle valve if needed. Adjustments should be made slowly as large changes in pressure will have a negative impact on the plasma circuits.
9. Treat the **polystyrene sheets** for 1 minute.
10. Turn OFF the plasma first, and then turn OFF the pump.
11. Slowly open the needle valve to vent chamber.
12. Take polystyrene sheets to fume hood for APTES treatment.

IV. Polystyrene APTES Treatment

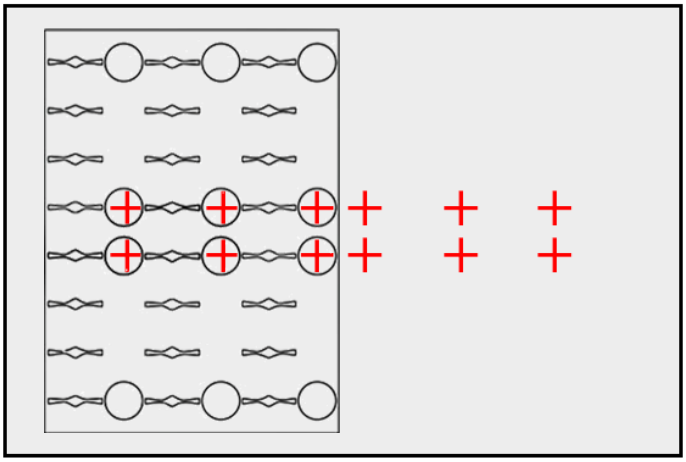
1. Prepare paper towels, a large beaker, and a glass tray for APTES treatment.
2. Transfer 900-1000 mL of DI water to the large beaker.
   This beaker will be used for later rinsing.
3. Prepare a 1% APTES solution to treat polystyrene sheets. *
   a. Measure 125 mL DI water in a beaker
   b. In the fume hood, use P1000 pipette to add 1.25 mL of APTES into the beaker.
   c. Mix the solution
4. Use tweezers to place the polystyrene sheets on the glass tray for soaking.
5. Slowly pour 1% APTES solution into the glass tray. Use tweezers to ensure that the polystyrene sheets are completely submerged.
6. Soak for 20 minutes.
7. Use tweezers to pick up polystyrene sheet. Wash polystyrene by slowly swirling in the large beaker with DI water. Afterwards, pat off excess water on a paper towel.
8. Place the polystyrene sheet on a drying rack and repeat for all sheets.
9. Allow polystyrene to dry in the fume hood for 10 minutes. **
10. After completing all desired treatments, transfer 1% APTES soak solution to waste container.
11. Rinse all glassware.
12. When dry, take drying rack with polystyrene sheets to bonding station.

** APTES solution must be prepared fresh for every treatment. Otherwise, bonding strength will be compromised. Multiple polystyrene sheets can be treated at once. The sheets can be stored for 30 minutes. If stored for longer, re-treatment may be necessary.*

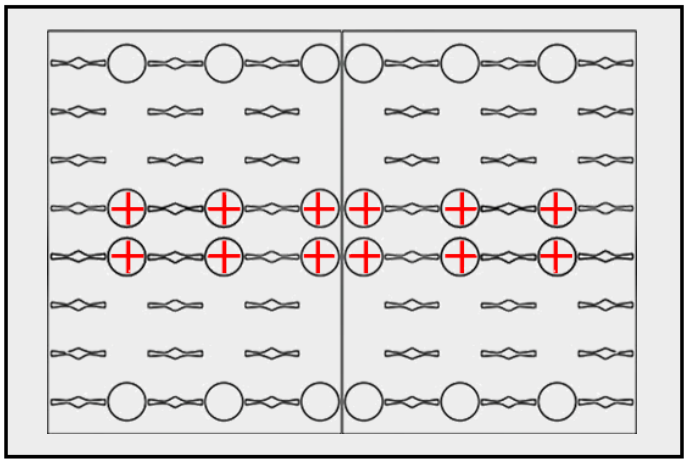
*** Longer times between APTES treatment and bonding will result in weaker bonds between polystyrene and PDMS. It is optimal to bond PDMS to the polystyrene sheet within 10-20 minutes after completing APTES treatment; otherwise, it will be necessary to re-treat the sheet(s).*

V. PDMS-Polystyrene Plasma Bonding

1. Use KimWipe to dry any remaining liquid on polystyrene sheets.
2. Repeat procedure outlined under **Polystyrene Plasma Pre-Treatment** to treat PDMS.
3. Treat one PDMS half for 2 minutes.
4. Slowly open the needle valve to vent chamber.
5. Bond PDMS to Polystyrene.
    a. Place polystyrene sheet on bottom. Tape down corners if necessary to minimize slippage during bonding.
    b. Line up guiding wells between the PDMS and the polystyrene sheet.



    c. Firmly push PDMS down onto the polystyrene sheet and hold for 2 minutes.
6. Repeat Steps 2-5 for a second PDMS half to finalize one device.



7. Clean up station after use.

VI. Primary Antibody Immobilization

Buffers Needed:

1. **Bicarbonate Coating Buffer**, pH to 9.6

2. **Blocking Buffer:**
   1% w/v BSA diluted in 1x PBS

3. **Wash Buffer:**
   0.05% v/v Tween-20 diluted in 1x PBS

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1. Prepare 5-10 μg /mL solution of primary antibody in bicarbonate coating buffer.
2. Coat micro-wells with primary antibody:
   a. Use a P200 pipette to draw up 8 μL of antibody in coating buffer.
   b. Push solution into micro-well.
      Stop when you can no longer see the outlines of the channels. As the solution enters the channels, the channels become transparent.
   c. Twist off the pipette tip.
   d. For the same micro-well, use the P200 pipette and an empty tip to aspirate the solution out from the other loading-pore.
      Stop when the liquid in both tips is about level.
   e. Repeat for all micro-wells.
3. Allow the device to incubate at room temperature for 30 minutes.
4. Remove pipette tips and aspirate out the solution for all micro-wells.
5. Wash each micro-well with 8 μL of wash buffer, 3 times each.
   Aspirate the wash buffer out of the channels in-between each wash.
6. Block with blocking buffer by following the same procedure outlined in **Step 2**.
7. Incubate the device at 4 degrees Celsius overnight.

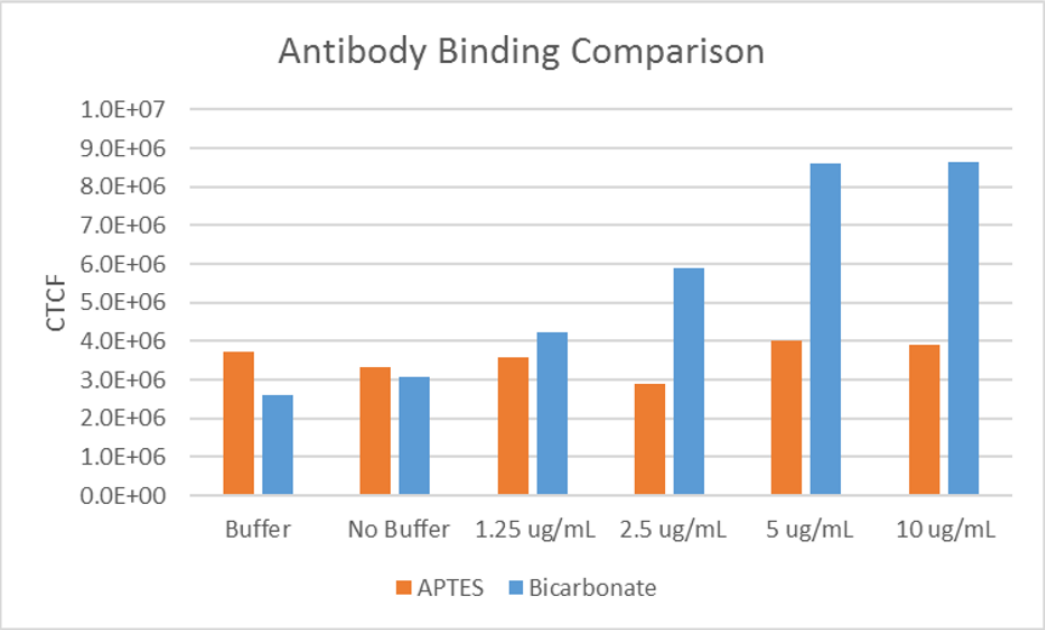
Antibody Binding Test

Goal

Determine which buffer, that is also readily available within the Hughes Laboratory, is most effective at binding antibodies to the device.

Additionally, determine whether the device is capable of ELISA as the technique requires the binding of antibodies.

Results



The results suggested that a **bicarbonate buffer yields the greatest binding strength** and confirmed that use of ELISA is possible.

Notes

Primary Antibody: Santa Cruz Biotechnology rabbit polyclonal sc-15402
Secondary Antibody: Alexa Fluor 488 anti-rabbit
Exposure time: 750 ms
Gain: 4

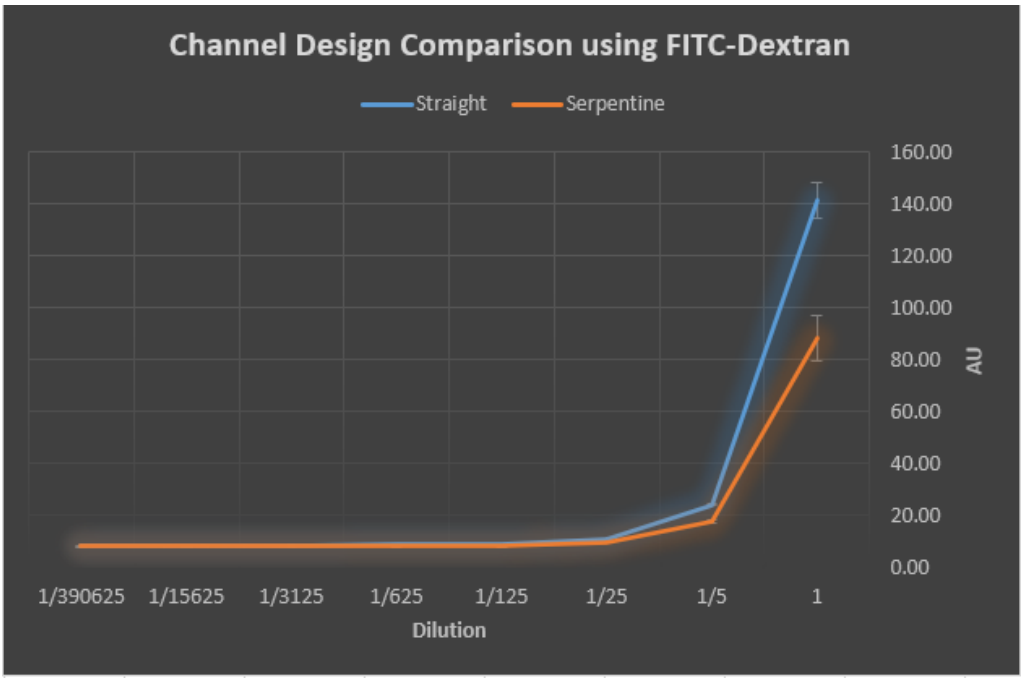
*Corrected Total Chamber Fluorescence (CTCF) = Intensity Density – (Area * BG Mean)*

Channel Comparison

Goal

Determine which channel design is more effective with the ELISA technique.

Results



Micro-wells with the straight channel returned greater absorbency readings, suggesting that the **straight design is more effective.**