Using Immunology as a Model to Teach Cell Communication

Including: A Practical ELISA For A Typical High School Biology Classroom

"Determination of a Standard Curve and Unknown Concentration of Monoclonal Antibody Using ELISA"

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Abstract:

This educational unit "Using Immunology as a Model to Teach Cell Communication" is developed for advanced high school biology classes (i.e. AP Biology or a second year course) to explore the inherent connections within the fields of Cell Communication and Immunology. This unit explores the processes involved in cell signaling, while simultaneously introducing the students to various levels of vertebrate immune response. Prior to completing the culminating laboratory activity, the students will gain knowledge of the two interrelated fields through a variety of lectures, written assignments, and a computer lab simulation. The final laboratory experiment, "*Determination of a Standard Curve and Unknown Concentration of Monoclonal Antibody Using ELISA*", was developed for a "typical" high school setting and budget; and was designed to complete a macro-scale ELISA (Enzyme-linked Immunosorbent Assay) using typical high school lab equipment. Upon conclusion of this curriculum, students will have developed a deeper understanding of not only Immunology and Cell Communication pathways, but also the concepts underlying an ELISA as a common lab assay used in the fields of immunology and medicine.

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Overview:

This curricular unit is designed to present students in a "typical" high school setting with the following aspects of Cell Biology and Immunology:

- An overview of cell-to-cell recognition using various cell surface molecules
- An overview of cell signaling
- Signal reception and the initiation of transduction
- Signal transduction pathways
- Cellular responses to signals
- Nonspecific defenses against infection based on cell signaling
- Specific immune responses based on cell signaling
- An overview of ELISA (Enzyme-Linked Immunosorbent Assay) assays and their importance
- Quantitative ELISA assay to determine standard and unknown concentrations of monoclonal antibodies (mAb)

The nonspecific and specific human immune responses function greatly on the concepts of cell-to-cell recognition and cell communication pathways. For this reason, the following unit was designed to connect cell-signaling details with the details of the human immune response, including the first, second, and third lines of defense. This unit is developed for Advanced Biology (i.e. a second year course or AP Biology) and should be taught during the instructor's Cell Biology portion of the curriculum.

Science Background:

-to

6. List and describe the nonspecific lines of defense in the vertebrate body
7. Distinguish between antigens and antibodies
8. Explain how B cells and T cells recognize specific antigens
9. Describe the mechanism of clonal selection and distinguish between effector and memory cells
10. Distinguish between the primary and secondary immune responses
11. Compare and contrast the structures and functions of cytotoxic T cells and helper T cells
12. Compare the production and functions of class I MHC and class II MHC molecules
13. Distinguish between humoral and cell-mediated immunity
14. Describe the functions of CD4, CD8, cytokines, perforin, interleukin-2 and interleukin-1
15. Diagram and label the structure of an antibody and explain how this structure allows antibodies to recognize and bind to antigens and assist in the destruction and elimination of antigens
16. Describe the production and uses of monoclonal antibodies
17. List some known autoimmune disorders and describe possible mechanisms of autoimmunity
18. Describe the basics of an ELISA assay and practical uses of this type of lab assay

Curriculum Unit Overview:

This curriculum unit includes lectures on cell communication topics and the human immune response. It also includes a computer ELISA lab simulation to be completed in class to introduce the students to the assay prior to the students completing an ELISA in the classroom laboratory, which is also included here. The unit strongly emphasizes the inherent connections between cell communication recognition, signaling, and the human immune system including nonspecific and

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Monoclonal Antibody Using ELISA"

Student Prior Knowledge:

Prior to completing the culminating laboratory activity of this unit connecting cell communication

IV. Immunity in Health and Disease
   a. Immunity can be achieved naturally or artificially
   b. The immune system's capacity to distinguish self from nonself limits blood transfusion and tissue transplantation
   c. Abnormal immune function can lead to disease
   d. Use of the common ELISA assay helps detect antibody levels in blood and urine tests

Curriculum Lessons:

Day #1:	Cell Communication PowerPoint/Lecture	
Day #2:	Cell Communication PowerPoint/Lecture/ Written Assignment	
Day #3:	Immunology PowerPoint/Lecture	
Day #4:	Immunology PowerPoint/Lecture	
Day #5:	Immunology PowerPoint/Lecture/ Written Assignment	
Day #6:	Howard Hughes Medical Institute Internet Lab Simulation	(see Appendix A)
Day #7, 8, 9, 10:	Laboratory Activity: *"Determination of a Standard Curve and Unknown Concentration of Monoclonal Antibody Using ELISA"*	(see "Part III: Laboratory Activity Details" and Appendix B)

Assessments:

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Using Immunology as a Model to Teach Cell Communication

Including: A Practical ELISA For A Typical High School Biology Classroom

"Determination of a Standard Curve and Unknown Concentration of Monoclonal Antibody Using an ELISA"

Time Requirements (overview):

Four class periods are needed for the lab protocol and analysis. (This is based on a 54-minute class period.)

Day #1: Preparation of control resin and MHCII-Resin with the primary antibody (i.e. MHCII monoclonal antibody)

Day #2: Introduction of the Secondary Antibody with enzyme attached (i.e. anti-mouse Ab-HRP)

Day #3: Introduction of enzyme substrate (i.e. TMB) and spectrophotometry

Day #4: Analysis: Class Data, Microsoft Excel scatter plot, linear determination of unknown antibody concentrations, and written analysis questions

Equipment List:

1. Clinical Table Top Centrifuge
2. Micropipettes and Tips
3. Spectrophotometer (required 450nm absorbance readings)
4. Orbital Shaker (optional but useful)
5. Vortex Mixer (optional)

Materials per Student Lab Group:

1. 4 – 1.5ml Microcentrifuge Tubes
2. 4 - 15ml Conical Tubes
3. Transfer Pipettes
4. Parafilm Wax (8- ½ inch strips)
5. Permanent Marker
6. Test Tube Rack-

Ordering Information:

Product	Company	Approximate Cost
Resin	GE Healthcare	$166.00 for 25ml
TMB Substrate	Sigma Scientific	$47.10 for 100ml
15ml conical tubes	USA Scientific	$124.00 for 500
1.5ml microcentrifuge tubes	USA Scientific	$30.00 for 500

Student Protocol:

(See the following pages for a copy of the student protocol. A copy of the student protocol, including analysis questions, is also provided in Appendix B for teacher originals for copying.)

Determination of a Standard Curve and Unknown Concentrations of Monoclonal Antibody Using ELISA

"A Practical ELISA For A Typical High School Biology Classroom"

Student Objectives:

1. Students will understand the concept and procedure of an enzyme-linked immunosorbent assay (ELISA).
2. Students will develop advanced lab skills.
3. Students will understand how to develop a standard curve using Microsoft Excel.
4. Students will understand how to determine unknowns from the standard curve using the Microsoft Excel software.
5. Student will develop an understanding of practical uses of ELISA assays in today's society.

Student Procedure Day #1:

1. Obtain 4 prepared microcentrifuge tubes (2 containing control resin, 2 containing cconjugated MHCII-Resin) from the teacher.

2. Hold the microcentrifuge tubes up to the light and confirm that each tube contains roughly the same amount of resin compared to the other tubes. (The resin has settled to the bottom.)

3. Using a sharpie, label the 4 microcentrifuge tubes:
    a. Label one control tube and one MHCII-Resin tube "Standard"
    b. Label the other control tube and MHCII-Resin tube "Unknown"
    c. Label ALL tubes with your initials and class period

4. Obtain the stock tube of your assigned "Standard" and "Unknown" from the teacher. Do NOT write on these tubes.

5. To each of the "Standard" tubes, add 135ul of the standard MHCII-mAb concentration assigned to your lab group (i.e. SF). Cap the tubes.

6. Change tips on the micropipette!

7. To each of the "Unknown" tubes, add 135ul of the unknown MHCII-mAb sample assigned to your lab group (i.e. UC). Cap the tubes.

8. Change tips on the micropipette!

9. Obtain a stock solution of 1xPBS-5%Skim Milk from the teacher

10. <u>You MUST change tips between each tube in this step</u>. To each of the 4 tubes, add 265ul of 1XPBS-5%Skim Milk solution, so that the total volume in each tube is now 500ul. Cap the tubes. Hold tubes up to confirm that the tubes have roughly the same amount of solution.

11. Invert the tubes gently mixing the solution. Also, flick the tip of each tube gently with your finger to re-suspend the resin into the solution.

12. Seal the 4 tubes with the small pieces of parafilm wax provided.

13. Place the 4 tubes on the orbital shaker for one hour. (The teacher will place tubes overnight in refrigerator after one hour of shaking.)

14. Obtain 4-15ml conical tubes with lids.

15. Label them accordingly:
    a. "Control Standard", the assigned code (i.e. SB), and your initials
    b. "Standard", the assigned code, and your initials
    c. "Control Unknown", assigned code, and initials
    d. "Unknown", assigned code, and initials

16. Place them in assigned tube rack until next day.

17. Properly clean up your lab station.

Student Procedure Day #2:

1. Pipette 10ml of 1XPBS-T (1X PBS-Tween) to each of the 4 conical tubes labeled at the conclusion of day #1.

2. You MUST change tips will each transfer. Transfer the 500ul of resin-MHCII-mAb in each of the 4 microcentrifuge tubes to the respective 15ml conical tube. Try to use the same tip with each tube to use a small amount of the 1X PBS-T to "rinse" out the microcentrifuge tube to minimize the amount of resin lost in the transfer. (The 4 microcentrifuge tubes get properly disposed.)

3. Cap and invert the conical tubes several times to resuspend the resin. (This is the wash step.)

4. Place the 4 tubes in the centrifuge, close lid, and spin tubes for 5 minutes at 1000 rpm.

5. You MUST use a new pipette for each tube. Using small transfer pipettes, carefully remove the supernatant from each tube. Dispose of each pipette after ONE use.

6. Add 10ml of 1XPBS-T to each conical tube.

7. Repeat steps #3-5 above (this is the second wash procedure).

8. Vortex the stock solution of anti-mouse Ab-HRP solution for a few seconds.

9. Add 1ml anti-mouse Ab-HRP (in 1XPBS) to each of the 4 conical tubes.

10. Cap tubes and gently invert tubes to resuspend resin in the anti-mouse Ab-HRP solution.

11. Place the 4 conical tubes on the orbital shaker for one hour. (The teacher will place tubes overnight in refrigerator after one hour of shaking.)

12. Properly clean up your lab station.

Student Procedure Day #3:

1. After removing tubes from the orbital shaker, add 10ml of 1XPBS-T to each conical tube.

2. Cap and invert the conical tubes several times to resuspend the resin. (This is the wash step.)

3. Place the 4 tubes in the centrifuge, close lid, and spin tubes for 5 minutes at 1000 rpm.

4. You MUST use a new pipette for each tube. Using small transfer pipettes, carefully remove the supernatant from each tube. Dispose of each pipette after ONE use.

5. Repeat steps #1-4 above (this is the second wash procedure).

6. <u>You MUST change tips between each tube.</u> Add 300ul TMB substrate to each of the 4 conical tubes. (The TMB substrate should be at room temperature.)

7. Cap tubes and gently shake by hand to mix.

8. Place the tubes on the orbital shaker for 20 minutes. (You should see a color change over this 20 minute time period.)

9. <u>You MUST change tips between each tube.</u> Add 300ul 1M HCl to each of the 4 conical tubes to stop the reaction between the HRP enzyme and the TMB substrate.

10. Cap the tube and gently shake by hand to mix.

11. Place the 4 tubes in the centrifuge, close lid, and spin tubes for 5 minutes at 1000 rpm.

12. <u>You MUST change tips between each tube.</u> Remove liquid supernatant to spectrophotometer cuvettes. ***Keep track of which cuvette has which standard or unknown solution, perhaps line them up in the test tube rack respectively, the cuvettes CANNOT be labeled. ***

13. Add 2.4ml of distilled water to each of the 4 cuvettes to dilute the samples for the spectrophotometer.

14. Calibrate the spectrophotometer with 3ml of distilled water in a separate cuvette.

15. Read and record (Table 1.1) the absorbance of each cuvette at 450nm. ***Be sure to keep track of which cuvette is which. ***

16. Properly clean up your lab station.

<u>Student Procedure Day #4:</u>

1. According to your teacher's instructions, collect class absorbance data for all the standard concentrations and the unknown concentrations. (Table 1.1)

2. Calculate class average absorbance for each standard and each unknown concentration.

3. Using Microsoft Excel, the absorbance values, and the scatter plot function; develop a standard curve for the 6 standard antibody concentrations. (Print the scatter plot.)

4. Using Microsoft Excel, linear extrapolation, and the scatter plot from #2 above, determine the concentration of the 4 unknown antibody concentrations. (Record in Table 1.2)

5. Answer the analysis questions.

All Lab Groups' Absorbance Differences and Averages:

ences:						
esult #2	Resu lt #3	Result #4				Average
esult #2	Result #3	Result #4	Result #5	Result #6		Average

l Analysis:

sing Excel develop a scatter plot for the class average absorbance of the 6 standard tibody concentrations. (See example)

dd a regression line into the scatter plot (i.e. a trend line).

ve your graph and data on the same page. Print this page.

sing the graphing calculators, find the equation of the line, and using the sorbance values (Y variable) determine the 4 unknown antibody ncentrations (X variable). Fill the values into the following table:

	Unknown A	Unknown B	Unknown C	Unknown D
nary ibody ntration				

[redacted]

D. Analysis Questions:

Directions – answer the following questions in complete sentences in the space provided.

1. Why is the control resin necessary?

2. Identify the following reagent by name:
    a. Primary Antibody =
    b. Secondary Antibody =
    c. Enzyme =
    d. Substrate=

3. Why is each standard and each unknown tests multiple times in all classes?

4. What is the purpose of washing the resin before adding each reagent?

5. What is the purpose of adding the 1M HCl at the end of Day #3? Explain

6. Explain what is meant by a false positive result. Name one error that would result in a false positive result.

7. Explain what is meant by a false negative result. Name one error that would result in a false negative result.

8. Using your ELISA knowledge, the textbook, and the Internet if needed, explain how this particular type of assay is issued to diagnose HIV, the virus associated with AIDS.

Teacher Protocol, Instructions, and Answer Keys:

Determination of a Standard Curve and Unknown Concentrations of Monoclonal Antibody Using An ELISA Assay

"A Practical ELISA Assay For A Typical High School Biology Classroom"

Overview:

This lab is meant to utilize typical high school lab department equipment and supplies to illustrate, on a macro-scale, an ELISA technique so that students are exposed to a common immunological lab experiment. Teacher preparation time is extensive, but materials can be used from year to year if kept in appropriate conditions. In addition, the cost of the resin, the primary antibody, secondary antibody, and substrate can seem somewhat large at first glance, but the materials, if stored properly, can be used for 5 to 10 years. This practical ELISA can be a great experience for your students and can certainly help students develop critical lab skills for future lab work (i.e. micropipetting, centrifugation, spectrophotometry, etc…).

Resin Conjugation:

- Need 200µl of MHCII-Resin and 200µl of control resin per lab group
- Need 2400µl (2.4ml) per class of 24 students in 12 lab groups

MHCII-Resin Conjugation and Control Resin Preparation:

1. Resin (NHS-activated Sepharose 4 Fast Flow) – *using 5 ml of resin and isopropyl alcohol in which it is stored.*
    a. Spin the 5ml in 15 ml Falcon tube down at 1000rpm, 5 minutes
    b. Pipette off alcohol supernatant (waste)
    c. Wash resin with 10-15 times volume of resin with cold 1mM HCl, invert tube to resuspend (*might need to was twice with smaller volumes, resuspend, spin, and remove supernatant each time*)
    d. Spin down each wash, 1000rpm, 5 minutes
    e. Remove wash supernatant (waste)
    f. Neutralize pH with 5ml 1M $NaHCO_3$ invert tube to resuspend
    g. Spin, 1000rpm, 5 minutes
    h. Remove supernatant (waste)

2. Mix MHCII into Resin (pipette in) **OR** If making "control resin," add a volume of ethanolamine to resin equal to the MHCII volume.

3. Add 400µl 2M NaCl

4. Add 400µl 1M $NaHCO_3$

5. Resuspend by inverting tube

6. Shake on orbital shaker in fridge (4C) overnight (2^{nd} option is shake at room temp for 2-4 hours)

7. After shaking overnight, block un-reacted groups
    a. Spin down coupled resin, 1000 rpm, 5 min.
    b. Remove supernatant (waste)
    c. Resuspend in 10ml 0.5M Ethanolamine
    d. Shake for at least 1.5 hours in room temperature

8. Wash Resin-MHCII Coupled Medium
    a. Spin down blocked medium and remove supernatant (waste)
    b. Alternate washes between 2 different buffers (high and low pH)
        i. 0.1M Tris-HCl pH 8-9
        ii.

4. **1M HCl**
    a. $M_1V_1 = M_2V_2$
    b. Example: Making 50ml of 1M HCl: (12M HCl)(X) = (1M HCl)(50ml), X=4.2ml

5. **1XPBS-5%Skim Milk**
    a. Add 50mg of powdered skim milk
    b. Add 1ml of distilled water
    c. Vortex mixture

Class Lab Group Organization:

This organization is based on a class of 24 students divided into 12 lab groups consisting of 2 students each. Each lab group will be assigned four microcentrifuge tubes. Two of the tubes will contain 200ul of control resin (100µl each), and two of the tubes will contain 200ul of conjugated resin (MHCII-Resin) (100µl each). The lab groups will also be assigned one standard antibody sample and one unknown antibody sample. The assignments are organized into Table 1.2.

Table 1.2

Lab Group	Tube #1 100µlControl Resin + Standard Sample	Tube #2 100µl MHCII-Resin + Standard Sample	Tube #3 100µl Control Resin + Unknown Sample	Tube #4 100µl MHCII-Resin + Unknown Sample
1	SA	SA	UA	UA
2	SB	SB	UB	UB
3	SC	SC	UC	UC
[redacted]				
5				

[redacted]

4. If possible, place the following lab equipment in a central locations easily accessible by all lab groups:
    a. Table top orbital shaker
    b. Clinical centrifuge(s)
    c. Vortex mixer
    d. Micropipettes (if not enough for every group)

5. Be sure to allow the TMB substrate to warm up to room temperature prior to use.

6. Be sure to allow the spectrophotometer to warm up for at least 15 minutes prior to use.

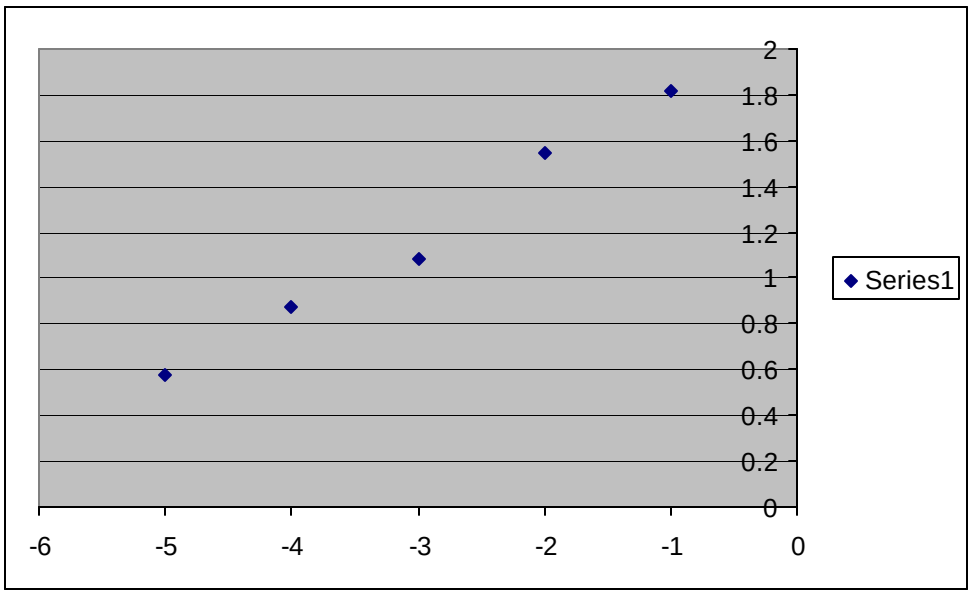
Standard Curve Preparation Suggestions:

1. When using Microsoft Excel, convert the standard antibody solutions to log10 in order to produce a scatter plot. See example below:

mAb	log10 [mAb]	Absorbance*
0.1	-1	1.82
0.01	-2	1.55
0.001	-3	1.08
0.0001	-4	0.87
0.00001	-5	0.58

* this is sample data for illustration purposes only

2. When using Microsoft Excel to develop the scatter plot, use the "Insert Chart" option and select "XY Scatter" as the type of chart to use. See example below based on above sample data:



3. To determine the unknown antibody concentrations, determine the line of regression for the scatter plot (either using Microsoft Excel or a graphing calculator) and obtain the equation of

Appendix A:
Howard Hughes Medical Institute
Biointeractive Lab Simulation
ELISA Computer Activity

Howard Hughes Medical Institute: Biointeractive Lab
Immunology Lab

Introduction:

1. Go to *www.hhmi.org/biointeractive/vlabs/*

10. Therefore, the amount of color reflects _____
_____.

Lab Notebook:
Proceed through the entire lab simulation protocol. Be sure and read captions below the pictures (left side) and the information in the lab notebook (right side).

Be sure to "start over" to begin the lab. You CANNOT skip any steps.

Answer the following questions as you proceed.

1. Define *Systemic Lupus Erythematosus (SLE)*.

2. From Figure 1 (click on it), what are the 4 Steps of an ELISA protocol?
   a.
   b.
   c.
   d.

3. What does a centrifuge do?

4. What are you preparing in Step 2?

5. (Step 3) What has the ELISA plate been pre-

13. (Step 7) What is the attached enzyme in this assay?

14. (Step 7) What is the specific substrate for HRP? What color does it produce?

15. (Step 10, in "why") How can the yellow color be quantitatively measured? At what wavelength?

16. **Results** (**Indicate on this page and on the computer which boxes turned colors**.)

	A	**B**	**C**	**+ (pos)**	**- (neg)**
1:2					
1:10					
1:100					

17. Did you complete the ELISA correctly? (Yes/No) _____

    If **yes**, proceed to #18.

    If **no**, proceed to #19.

18. What do the results indicate about:
    Patient A _____
    Patient B _____
    Patient C _____

19. Explain what you did wrong and what you will need to do next time. Did your incorrect procedure provide you any results? Explain what went wrong.

Appendix B:
Student Lab Protocol

Determination of a Standard Curve and Unknown Concentrations of Monoclonal Antibody Using ELISA

“A Practical ELISA Assay For A Typical High School Biology Classroom”“nh- uoT 7n ( ) Tj Br

13. Place the 4 tubes on the orbital shaker for one hour. (The teacher will place tubes overnight in refrigerator after one hour of shaking.)

14. Obtain 4-15ml conical tubes with lids.

15. Label them accordingly:
    a. "Control Standard", the assigned code (i.e. SB), and your initials
    b. "Standard", the assigned code, and your initials
    c. "Control Unknown", assigned code, and initials
    d. "Unknown", assigned code, and initials

16. Place them in assigned tube rack until next day.

17. Properly clean up your lab station.

Student Procedure Day #2:

1. Pipette 10ml of 1XPBS-T (1X PBS-Tween) to each of the 4 conical tubes labeled at the conclusion of day #1.

2. You MUST change tips will each transfer. Transfer the 500ul of resin-MHCII-mAb in each of the 4 microcentrifuge tubes to the respective 15ml conical tube. Try to use the same tip with each tube to use a small amount of the 1X PBS-T to "rinse" out the microcentrifuge tube to minimize the amount of resin lost in the transfer. (The 4 microcentrifuge tubes get properly disposed.)

3. Cap and invert the conical tubes several times to resuspend the resin. (This is the wash step.)

4. Place the 4 tubes in the centrifuge, close lid, and spin tubes for 5 minutes at 1000 rpm.

5. You MUST use a new pipette for each tube. Using small transfer pipettes, carefully remove the supernatant from each tube. Dispose of each pipette after ONE use.

6. Add 10ml of 1XPBS-T to each conical tube.

7. Repeat steps #3-5 above (this is the second wash procedure).

8. Vortex the stock solution of anti-mouse Ab-HRP solution for a few seconds.

9. Add 1ml anti-mouse Ab-HRP (in 1XPBS) to each of the 4 conical tubes.

10. Cap tubes and gently invert tubes to resuspend resin in the anti-mouse Ab-HRP solution.

11. Place the 4 conical tubes on the orbital shaker for one hour. (The teacher will place tubes overnight in refrigerator after one hour of shaking.)

12. Properly clean up your lab station.

Student Procedure Day #3:

1. After removing tubes from the orbital shaker, add 10ml of 1XPBS-T to each conical tube.

2. Cap and invert the conical tubes several times to resuspend the resin. (This is the wash step.)

3. Place the 4 tubes in the centrifuge, close lid, and spin tubes for 5 minutes at 1000 rpm.

4. You MUST use a new pipette for each tube. Using small transfer pipettes, carefully remove the supernatant from each tube. Dispose of each pipette after ONE use.

5. Repeat steps #1-4 above (this is the second wash procedure).

6. You MUST change tips between each tube. Add 300ul TMB substrate to each of the 4 conical tubes. (The TMB substrate should be at room temperature.)

7. Cap tubes and gently shake by hand to mix.

8. Place the tubes on the orbital shaker for 20 minutes. (You should see a color change over this 20 minute time period.)

9. You MUST change tips between each tube. Add 300ul 1M HCl to each of the 4 conical tubes to stop the reaction between the HRP enzyme and the TMB substrate.

10. Cap the tube and gently shake by hand to mix.

11. Place the 4 tubes in the centrifuge, close lid, and spin tubes for 5 minutes at 1000 rpm.

12. You MUST change tips between each tube. Remove liquid supernatant to spectrophotometer cuvettes. ***Keep track of which cuvette has which standard or unknown solution, perhaps line them up in the test tube rack respectively, the cuvettes CANNOT be labeled. ***

13. Add 2.4ml of distilled water to each of the 4 cuvettes to dilute the samples for the spectrophotometer.

14. Calibrate the spectrophotometer with 3ml of distilled water in a separate cuvette.

15. Read and record (Table 1.1) the absorbance of each cuvette at 450nm. ***Be sure to keep track of which cuvette is which. ***

16. Properly clean up your lab station.

Student Procedure Day #4:

1. According to your teacher's instructions, collect class absorbance data for all the standard

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A. Raw Data: All Lab Groups Absorbance Reading and Calculated Absorbance Differences:

Period	Group Number	Standard	MHCII-Resin	Control - Resin	Absorbance	Unj0.2895 Tw ( ) Tj ET Qbsorbance

B. Compiled Data: All Lab Groups' Absorbance Differences and Averages:

Absorbance Differences:								
	Result #1	Result #2	Result #3	Result #4				Average
SA								
SB								
SC								
SD								
SE								
SF								

D. Analysis Questions:

Directions – answer the following questions in complete sentences in the space provided.

1.