

EDVOTEK WORKSHOP:

# Diagnosing the Silent Killer: A Simulation of the Clinical Detection of Diabetes



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## Background Information

Excerpts from Edvokit #280

### Introduction

According to the International Diabetes Federation (IDF), an estimated 382 million individuals are living with diabetes in 2013, and that number is expected to nearly double by 2035. It is important to effectively diagnose and treat the disease early because increased levels of sugar in the blood causes serious complications if left untreated. Participants distinguish between the two main types of diabetes using urinalysis and ELISA. Receive a 4GB flashdrive and be entered for a T-shirt drawing.

### Detecting the "Silent Killer": A Simulation of the Clinical Detection of Diabetes

Diabetes mellitus, commonly called the "Silent Killer", is a chronic disease that leads to increased levels of sugar (glucose) in the blood. It is estimated that 8.3% of the United States population is diabetic, of which 7 million are undiagnosed. In addition, this disease affects millions of people around the world, both young and old.

Blood sugar levels are regulated by insulin, a hormone that is synthesized in and secreted by the beta cells in the pancreas. Mature insulin consists of two distinct protein chains that are linked by disulfide bonds. However, insulin is initially synthesized as a single protein chain called preproinsulin, which consists of four domains: a signal peptide, the carboxyl-terminal a chain, the amino-terminal B chain, and the C-peptide that connects the two terminals (Figure 1). The signal peptide at the amino terminal facilitates the transport of preproinsulin into the endoplasmic reticulum to process insulin. Within the endoplasmic reticulum, enzymes known as signal peptidases remove the signal peptide to form what is called, proinsulin (Figure 1A). Subsequently, disulfide bonds form between the A and B chains. The next cleavage occurs by enzymes called endopeptidases that remove the C-peptide from proinsulin (Figure 1B), producing the mature form of insulin (Figure 1C).

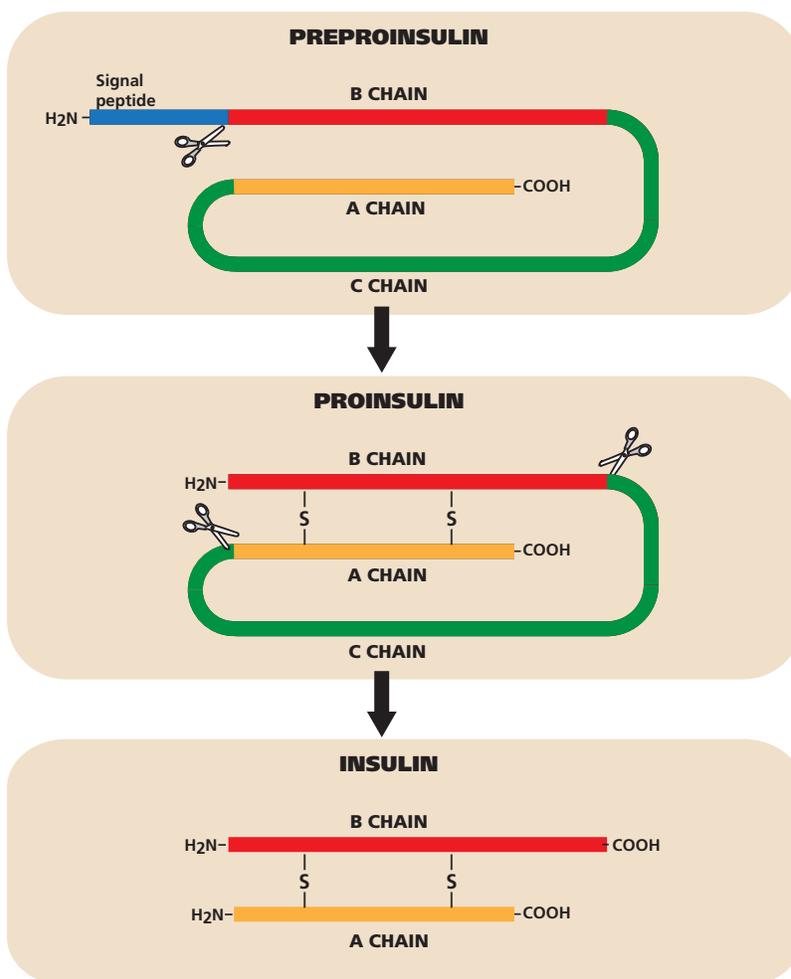


Figure 1: Maturation of Insulin

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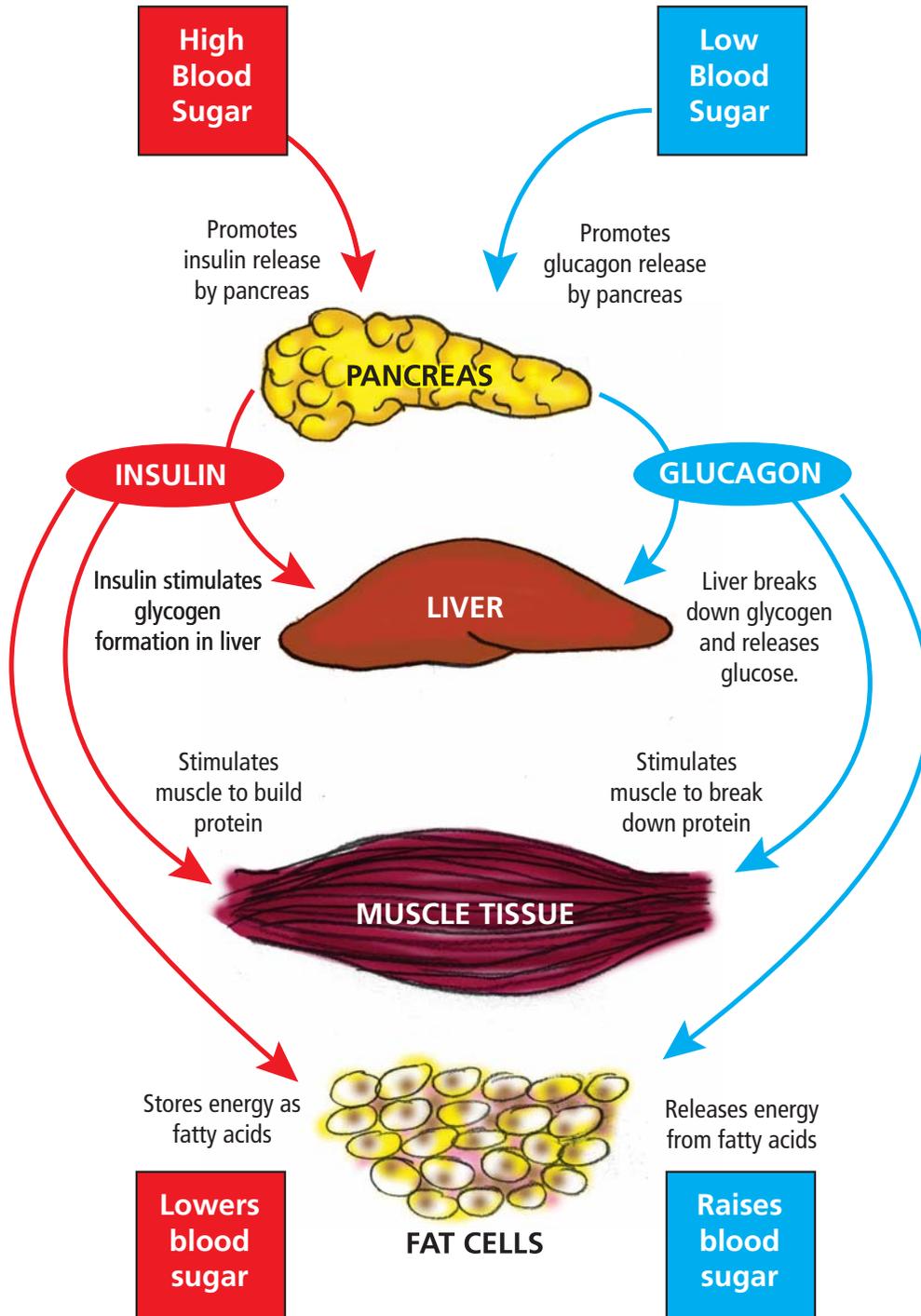


Figure 2: Glucose Homeostasis

## Background Information

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In healthy individuals, insulin is stored in the pancreas until blood glucose levels begin to rise (e.g. after a meal). At this time, the insulin is secreted (Figure 2). Once in the blood stream, insulin signals the surrounding cells to import glucose (Figure 3A). The cells immediately break down some of glucose to produce energy. Excess glucose is generally stored in one of two forms: glycogen and triacylglycerols. Glucose monomers can assemble into a branched polysaccharide molecule called glycogen, which is stored in liver and muscle tissues. Excess glucose stimulates the conversion of free fatty acids to triacylglycerols, which are stored as fat in adipose tissue. When glucose levels are low, the hormone glucagon stimulates the release of the stored energy from the liver, muscle, and adipose tissues (Figure 2).

Diabetes occurs when the body becomes unable to regulate the level of glucose in the blood. The most common forms of diabetes are Type 1 and Type 2 diabetes. Type 1 diabetes, also known as juvenile or insulin-dependent diabetes, occurs in childhood and is more severe. In Type 1 diabetes, the beta cells undergo autoimmune destruction progressively and the pancreas releases little or no insulin (Figure 3b). Due to this, the body starts relying on energy derived from the breakdown of fats stored in the adipose cells. Ketones are formed as byproducts of the fat being metabolized. An excess of ketones in the body results in a condition known as ketoacidosis, which can lead to a diabetic coma, or even death. To prevent this, patients with Type 1 diabetes rely on daily injections of insulin for proper glucose metabolism. Some of the symptoms of Type 1 diabetes include excessive thirst (polydipsia), frequent urination (polyuria), high blood sugar (hyperglycemia), fatigue and weight loss.

Type 2 diabetes occurs when the body becomes resistant to insulin, or if the pancreas is not producing enough insulin (Figure 3C). The symptoms of type 2 diabetes are similar to that of Type 1, but they often go unrecognized because of the slow progression of the disease. Individuals affected by Type 2 diabetes may have a genetic pre-disposition to diabetes, but environmental factors—such as an unhealthy lifestyle—also trigger the development of this life-long disease. Due to sedentary lifestyles and the convenience of fast food restaurants, many overweight adults (>25 BMI) over the age of 40 are at a high risk of developing Type 2 diabetes. Although some patients with Type 2 diabetes can regulate the condition with lifestyle changes, most will rely on daily insulin injections to metabolize glucose.

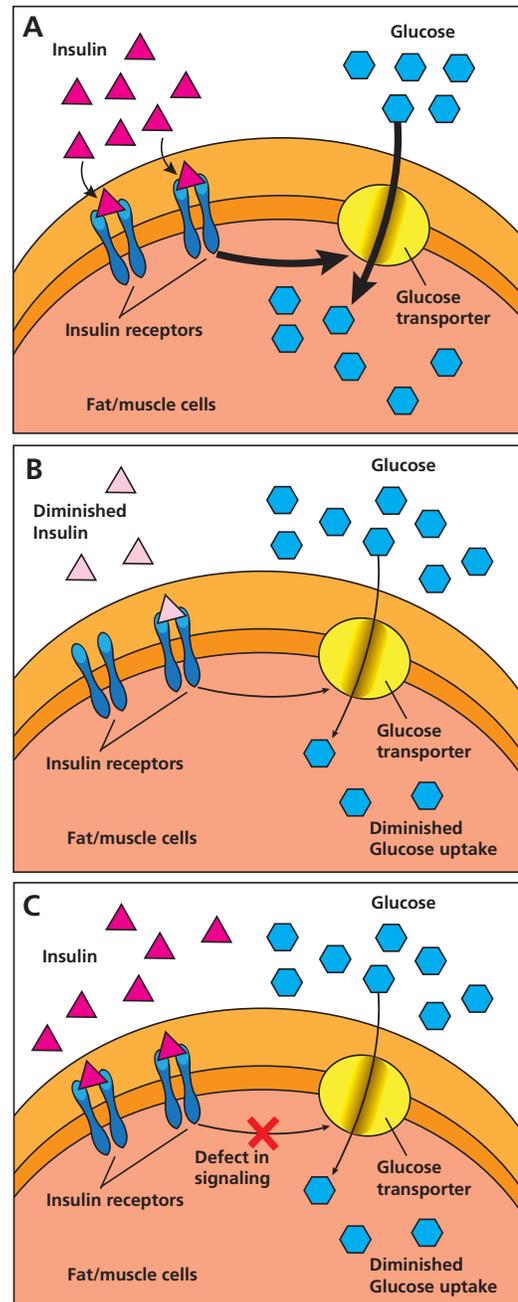


Figure 3: The effect of insulin levels on glucose uptake.

## Background Information

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### Diagnosing Diabetes

If a person experiences symptoms that suggest diabetes, a physician first will determine whether the patient has high blood sugar. Blood glucose levels can be monitored with a simple urine test, as high levels of glucose cannot be reabsorbed by the kidney. As such, excess glucose is excreted into the urine (glucosuria). The patient's urine sample is analyzed using a reagent that undergoes a chemical conversion in the presence of glucose and/or ketones, producing a dramatic color change. The final color of the sample indicates the level of glucose present in the urine (Figure 6). A healthy individual will have a fasting glucose level of around 75-100 milligrams per deciliter (mg/dL). If the urine test indicates an elevated level of sugars and ketones (usually around 125 mg/dL), blood is drawn and sent for further analysis before a formal diagnosis is made.

The Enzyme-Linked Immunosorbent Assay (or ELISA) is commonly used to differentiate between Type I and Type II diabetes. This assay uses antibodies to detect the presence of specific biomolecules (i.e. peptides, proteins, antigens and hormones) in a complex sample (Figure 7). Since the ELISA is highly sensitive, it is able to detect the C-peptide in a healthy patient's blood sample. Since a patient with Type I diabetes will not produce any insulin, the C-peptide is not present in the blood sample. In contrast, patients with Type II diabetes still produce a low amount of insulin, so the test will detect low amounts of the C-peptide in the blood.

The ELISA is performed in a transparent plastic microtiter plate that contains many small wells. The sample to be analyzed is added to the wells of the microtiter plate, where it non-specifically adheres to the plastic through hydrophobic and electrostatic interactions. Any unbound sample is washed out with a non-reacting buffer. The wells are then "blocked" with a protein-containing buffer (commonly casein or bovine serum albumin) to prevent non-specific interactions between the antibodies and the plastic wells. The next step is the addition of the primary antibody to the wells. The primary antibody recognizes a specific area of the antigen (called an epitope) and non-covalently binds. After the incubation period, the wells are washed to remove any primary antibody that did not bind.

Following the blocking step, a purified secondary antibody is added. The secondary antibody is a polyclonal antibody that recognizes and binds to the primary antibody. For example, if a rabbit produced our primary antibody, we would use a secondary antibody that recognizes rabbit antibodies. Once the secondary antibody has attached to the primary antibody, any unbound antibody is washed away with buffer.

The secondary antibody is covalently linked to an enzyme like Horseradish Peroxidase (HRP) that allows for the detection of the antibody-antigen complex. A clear, colorless substrate solution, containing hydrogen peroxide and amino salicylate is added to each well. In wells where the secondary antibody is present, the HRP will convert hydrogen peroxide to  $H_2O + O_2$ , which in turn oxidizes the amino salicylate. Since oxidized amino salicylate is brown in color, the solution in wells that contain the antibody-antigen complex will change from colorless to brown. HRP has a high catalytic activity, with its substrate turnover rates exceeding 106 per second, allowing us to quickly detect even the smallest amount of antigen.

The following experiments simulate the medical testing performed by doctors to diagnose Type I or Type II Diabetes. In the first part of this experiment (Module I), students will receive simulated urine samples of patients and use a chemical reagent to distinguish between the diabetic and non-diabetic condition. In the second part of the experiment (Module II), students will differentiate between Type I and Type II Diabetes.

|      |            |
|------|------------|
|      | Negative   |
| 1/10 | 100 mg/dL  |
| 1/4  | 250 mg/dL  |
| 1/2  | 500 mg/dL  |
| 1    | 1000 mg/dL |
| 2 +  | 2000 mg/dL |

Figure 4: Blood Glucose Levels

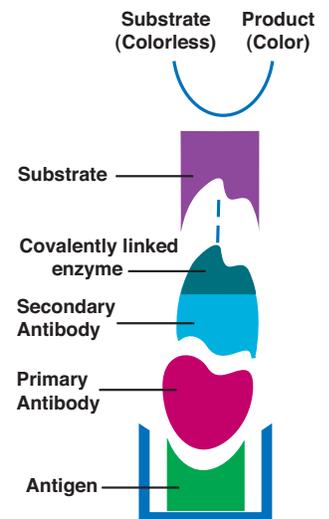


Figure 5: Schematic of traditional ELISA with primary and secondary antibodies.

## Experimental Procedure

Excerpts from Edvokit #280

**REVIEW** the Patient History and **RECORD** the symptoms related to Diabetes in the table (below) **BEFORE** performing the experiment.

- Patient A 50 yr old male, average weight, symptoms include excessive urination. Patient is very active. Last physical indicated high blood pressure.
- Patient B 12 yr old female, under weight, symptoms include excessive thirst and dramatic weight loss. Parents report the child often falls asleep in class.
- Patient C 50 yr old male, overweight, symptoms include excessive urination. Patient is sedentary. Last physical indicated borderline high blood sugar.

| Sample    | Symptoms | Urinalysis | ELISA | Diagnosis |
|-----------|----------|------------|-------|-----------|
| Patient A |          |            |       |           |
| Patient B |          |            |       |           |
| Patient C |          |            |       |           |

**NEW**

*Coming This Fall...*

### Clinical Detection of Diabetes

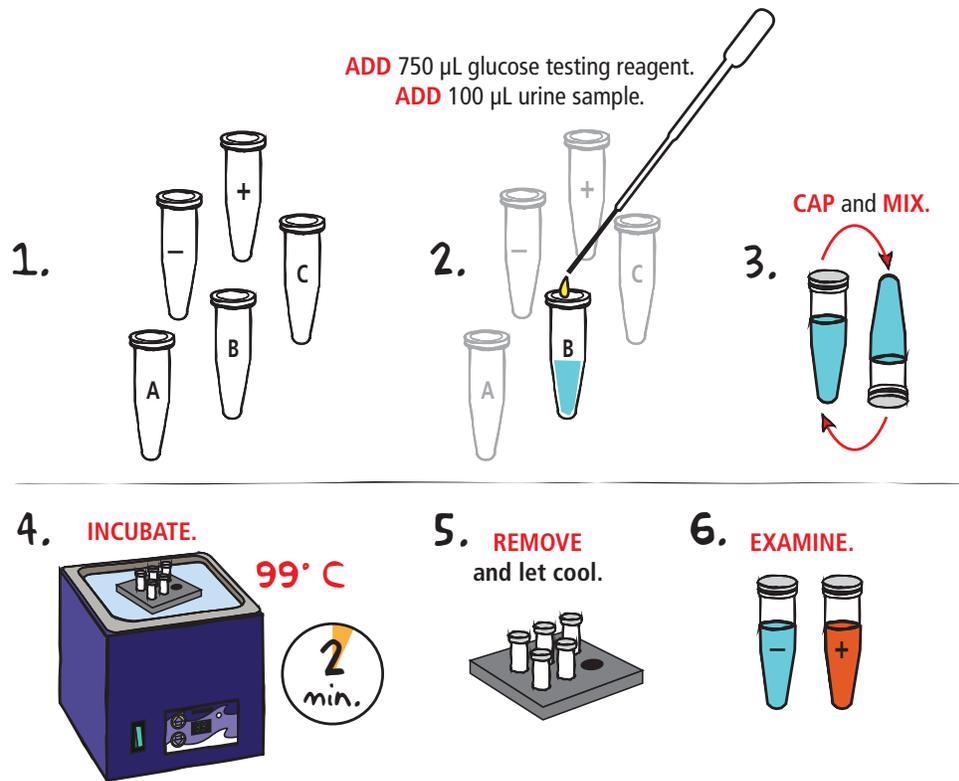
Diabetes mellitus is a chronic disease that leads to increased levels of sugar (glucose) in the blood. It is important to effectively diagnose and treat the disease early because increased levels of sugar in the blood causes serious complications if left untreated. The most common forms of diabetes are Type 1 (juvenile) and Type 2 (adult-onset) diabetes. In this experiment, students will between the two main types of diabetes using urinalysis and ELISA.



Cat. #280 \$139

# Urine Glucose Test

Excerpts from Edvokit #280



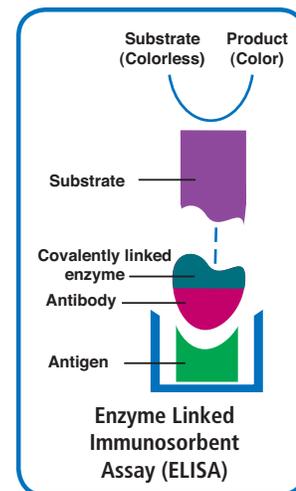
- LABEL** the microcentrifuge tubes as follows:
  - (-) negative control
  - (+) positive control
  - (A) patient A
  - (B) patient B
  - (C) patient C
- ADD** 750  $\mu\text{L}$  glucose testing reagent to each tube. Then, **ADD** 100  $\mu\text{L}$  of each urine sample to the appropriately labeled tube.
- SCREW** the lids on tightly. **MIX** the samples by inversion.
- INCUBATE** the samples in a 99°C water bath for two minutes.
- Carefully **REMOVE** the samples from the water bath and place them on your bench to cool.
- EXAMINE** the samples. Negative samples will remain blue, while positive samples will turn brick red. **RECORD** your results in table on page 6.

**NOTE:** A brownish precipitate may appear in the samples. Mix the samples well and proceed with the data analysis. This will not interfere with the results.

# C-peptide Detection by ELISA

Excerpts from Edvokit 267

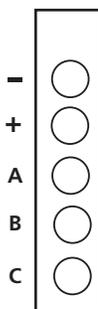
The ELISA protocol has been modified to fit into the workshop. The microtiter wells are pre-treated with the antigen. After the simulated patient sample is added to the wells, washed, and substrate is added, the conversion of the substrate to product results in the color formation for positive samples.



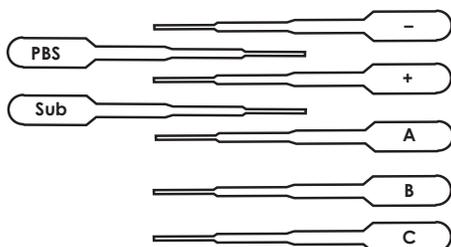
## LABEL MATERIALS:

- 1** Label wells: "-", "+", "A", "B", and "C" directly on the microtiter plate, or place the plate on a labeled sheet of paper.

Put your initials or group number on the plate.



- 2** Label pipets: "-", "+", "A", "B", and "C". These are designated for adding samples and removing washes - save these pipets!



- 8** Incubate for 10 minutes.



- 9** Remove all liquid using the transfer pipet designated for each row.

- 10** Wash each well with PBS buffer by adding the PBS buffer until each well is almost full. The capacity of each well is approximately 0.2 ml. Do not allow the liquids to spill over into adjacent wells. Remove all the PBS from each of the wells with the transfer pipet designated for each row.

## SUBSTRATE:

- 11** Add 0.1 ml or 5 drops of the substrate solution to all of the wells.

- 12** Incubate for 10 minutes.



## ANTIBODY CONTROLS AND SAMPLES:

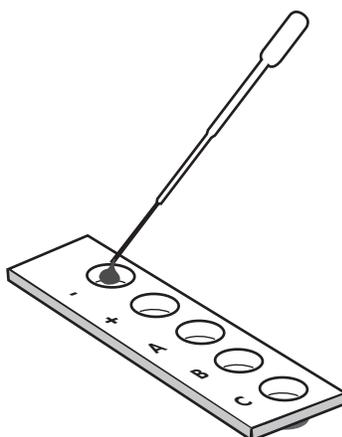
- 3** Add 3 drops of Negative Control to all three wells in the 1st Row.

- 4** Add 3 drops of Positive Control to all three wells in the 2nd Row.

- 5** Add 3 drops of Patient Sample A to all three wells in the 3rd Row.

- 6** Add 3 drops of Patient Sample B in all three wells in the 4th Row.

- 7** Add 3 drops of Patient Sample C in all three wells in the 5th Row.



## ANALYSIS:

- 13** Remove the plate for analysis.

If color is not fully developed after 10 minutes (step 12), incubate at 37°C for a longer period of time.

## Results and Discussion

| Sample    | Symptoms   | Urinalysis | ELISA | Diagnosis |
|-----------|--|------------|-------|-----------|
| Patient 1 | Frequent urination                                       | Neg        | Pos   | Neg       |
| Patient 2 | Thirst, weight loss, fatigue                             | Pos        | Neg   | Type I    |
| Patient 3 | Overweight, freq. urination, sedentary, high blood sugar | Pos        | Pos   | Type II   |

## Related Products



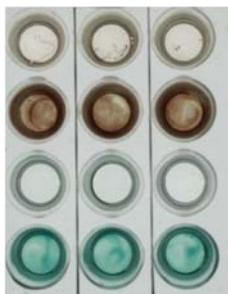
### Single Antibody ELISA Diagnostics

Teach your students the ELISA technique in less than half the time of traditional ELISAs! This experiment eliminates the need for the primary and secondary antibody normally needed for ELISAs because the detection antibody has an enzyme linked to it directly. Simply add substrate to discover which patient is infected.

-  For 10 Lab Groups
-  Complete in 20 min.
-  Cat. #267    \$99

**Kit includes:** instructions, antigens & antibodies, substrate, phosphate buffered saline, tubes, plates, and transfer pipets.

**All you need:** distilled or deionized water, 37° C incubation oven, automatic micropipets with tips, laboratory glassware.



### Introduction to ELISA Reactions

Your students will learn the basic principles of the Enzyme-linked Immunosorbent Assay (ELISA) in this precise and sensitive antibody-based detection kit. Experiment components do not contain human serum.

-  For 10 Lab Groups
-  Complete in 45 minutes
-  Cat. #269    \$99

**Kit includes:** instructions, antigens, primary & secondary antibodies, peroxide co-substrate, hydrogen peroxide, ABTS substrate, phosphate buffered saline, tubes, plates, and transfer pipets.

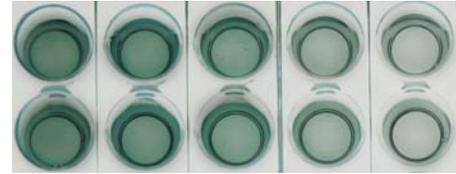
**All you need:** distilled or deionized water, 37° C incubation oven, automatic micropipets with tips, laboratory glassware.



## Related Products

### Quantitative ELISA Laboratory Activity

Antibodies are highly specific in their recognition of antigens. This ELISA experiment demonstrates the quantitation of varying concentrations of viral antigens as detected by the intensity of the color reaction due to the accumulation of products. This laboratory activity meets the requirements in the BSCS Blue Biology curriculum.



 For 6 Lab Groups

 Complete in 2 hours

 Cat. #278 \$99

**Kit includes:** instructions, antigens, primary & secondary antibodies, substrate solution, phosphate buffered saline, blocking agent, stop solution, tubes, plates, and transfer pipets.

**All you need:** distilled or deionized water, 37° C incubation oven, automatic micropipets with tips, laboratory glassware.

### Radial Immunodiffusion

Radial immunodiffusion quantitatively determines the level of an antigen. Antibody is incorporated into liquefied agar and allowed to gel. The antigen is added to small wells and radiates throughout the antibody-containing medium, leaving a precipitate throughout the gel. The amount of amount of diffusion is quantified.



 For 10 quantifications  
6 reactions each

 Incubation overnight

 Cat. #273 \$105

**Kit includes:** instructions, antigen and antibody, petri plates, pipets, well cutters, agarose, buffer, microtest tubes.

**All you need:** automatic micropipets with tips, waterbath, microwave or hot plate, incubation oven, laboratory glassware, pipet pumps or bulbs, rulers, paper towels, distilled water.



### EdvoPette™ Pipet Controller

The all-new EdvoPette™ Pipet Controller is a lightweight cordless pipetting controller ideally suited as an aliquoting tool for instructors and teaching assistants. It utilizes all standard serological pipets. The speed can be fine-tuned by applying varying finger pressure to the operating buttons.

 Cat. # 594 \$315



### Incubation Oven

This economical bacterial incubator features a digital temperature control with a range from Ambient +1° C to 60° C. Ideal for growing bacteria on agar plates at 37° C or for Southern and Western Blot analysis at 60° C. Includes two adjustable/removable shelves for increased capacity. Accepts bottles and flasks up to 2 L.

 Cat. #546 \$429



### Mini EdvoRokr™

The Mini EdvoRokr™ features a tilt angle and optimized speed for gel blotting, washing and staining. With the tri-directional motion, the rocker provide thorough and gentle mixing ability. The 10.5" X 7.5" autoclavable plat mat can accept stackable platforms and are safe to use in cold rooms and incubators (4°C to 65°C).

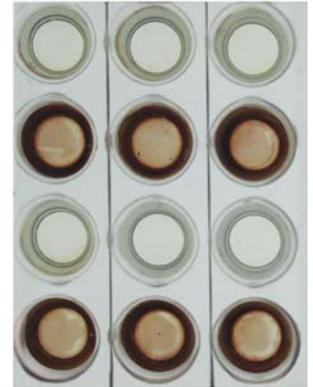
 Cat. #5019 \$379

## Related Products

### Simulation of HIV Detection by ELISA



An HIV test detects HIV infection indirectly using an ELISA test against HIV antibodies in the blood. The test works by taking antibodies from the patient's blood and adding them to a microtiter plate coated with HIV antigen. If HIV antibodies are present in the blood, they will bind to the antigens on the plate. This binding is detected with an enzyme-linked secondary antibody that causes a color change upon addition of substrate. In this experiment, your students will perform an ELISA test by coating microtiter plate wells with simulated HIV antigen and then test simulated donor serum for anti-HIV antibodies.



-  For 10 groups
-  Requires 1 hour
-  Cat. #271    \$99

**Kit includes:** instructions, serum samples, antigens and antibodies, various solutions, microtiter plates, various pipets and microtest tubes.

**All you need:** 37°C incubation oven, automatic micropipets with tips, pipet pumps, laboratory glassware, distilled or deionized water.

### In Search of the “Kissing Disease”

Infectious mononucleosis is commonly known as the “kissing disease”. The causative agent is Epstein-Barr virus (EBV) which can be transmitted through saliva during kissing. In this experiment, students search for the presence of EBV using the ELISA reaction to detect specific viral proteins.

-  For 10 groups
-  Requires 50 min.
-  Cat. #274    \$99

**Kit includes:** instructions, samples, antigens & antibodies, various solutions and reagents, pipets and microtest tubes.

**All you need:** 37°C incubation oven, automatic micropipets with tips, laboratory glassware, distilled or deionized water.



### Immunology of Pregnancy Tests

One of the most commonly used over-the-counter diagnostic tests is the pregnancy test, based on the Enzyme-linked Immunosorbent Assay (ELISA). The experimental concepts and methodology involved with the ELISA will be introduced in the context of testing for pregnancy. None of the components have been prepared from human sources.

-  For 10 groups
-  Set up 60 min.  
Experiment 60 min.
-  Cat. #279    \$99

**Kit includes:** instructions, samples, antibodies, various solutions and reagents, microtiter strips, pipets and tubes.

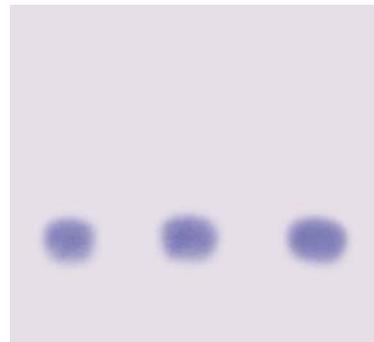
**All you need:** 37°C incubation oven, automatic micropipets with tips, laboratory glassware, distilled or deionized water.



## Related Products

### HIV Detection by Simulated Western Blot

The second assay used to confirm a positive HIV ELISA result is the Western Blot. Students separate protein samples from hypothetical patients on agarose gels, transfer the samples to a membrane and detect the simulated HIV proteins. This kit is an introductory level experiment. For a comprehensive advanced course, we recommend Cat. #317.



 For 6 Blots

**Kit includes:** instructions, samples, standard molecular weight markers, protein agarose, various buffers and reagents, PVDF membrane, filter paper, stain, 1 ml pipet, 100 ml graduated cylinder.

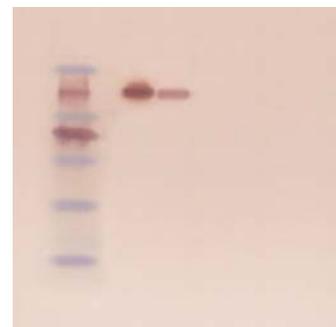
 Prep & Electroph. 1.5 hrs.  
Blot overnight  
Detection 25 min.

**All you need:** electrophoresis apparatus, power supply, automatic micropipets with tips, microwave or hot plate, incubation oven, shaker platform, lab glassware, small plastic trays, microtest tubes, pipet pumps or bulbs, metric rulers, distilled water, isopropanol, glacial acetic acid.

 Cat. #275    \$99

### Western Blot Analysis

In Western blot analysis, protein identification is based on antibody and antigen reactions. Proteins are separated on a polyacrylamide gels and are transferred (blotted) to a nylon membrane. The membrane is exposed to solutions containing primary antibody, followed by a secondary antibody coupled to an enzyme. The membrane is then soaked in a substrate solution to develop the color reaction, which results in identification of the antigen protein band. The molecular weights of the visible bands are measured using prestained protein markers of known molecular weight. This kit does not require an electrotransfer apparatus.



 For 6 Blots

**Kit includes:** instructions, negative control, all samples & antibodies, various reagents and buffers, membrane and filter paper.

 Electrophoresis 60 min.  
Blot overnight  
Detection 2.5 hours

**All you need:** 3 polyacrylamide gels (12%), Vertical gel electrophoresis apparatus, power supply, automatic micropipet with fine tips, laboratory glassware, metric rulers, distilled or deionized water, glacial acetic acid, methanol.

 Cat. #317    \$199

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Mon-Fri  
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Please Have the Following Info:

- Experiment number and title
- Kit lot number on box or tube
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