

Biotechnology In Your Mouth

(BE-102)

MATERIALS INCLUDED WITH THE KIT

This kit has enough materials and reagents for 30 students (six groups of five students).

Checklist

- 1 bottle Protein Dye Solution
- 10 Large Transfer Pipettes
- 80 Small Transfer Pipettes
- 1 bottle Enzyme Color Dye-I
- 1 vial Enzyme Color Dye-II
- 1 bottle Enzyme Substrate (starch)
- 6 Petri Dishes
- 1 bottle LB Agar Premade
- 30 Inoculating Loops
- 180 Centrifuge Tubes (2ml)

SPECIAL HANDLING INSTRUCTIONS

- All reagents can be stored at room temperature.

The majority of reagents and components supplied in the *BioScience Excellence™* kits are non toxic and are safe to handle, however good laboratory procedures should be used at all times. This includes wearing lab coats, gloves and safety goggles.

For further details on reagents please review the Material Safety Data Sheets (MSDS). The following items need to be used with particular caution.

Part #	Name	Hazard
E141	Enzyme Color Dye-I	Corrosive

ADDITIONAL EQUIPMENT REQUIRED

- Waterbath or beaker and thermometer
- Incubator (Optional)

TIME REQUIRED

- **Day 1:** 2-3 hours
- **Day 2:** 20 minutes



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OBJECTIVES

- Visualize the proteins in your saliva.
- Use a digestive enzyme in your saliva for a colorful assay.
- Grow your own bacteria that live in your mouth.

BACKGROUND

Biotechnology is one of the newest and fastest growing scientific fields that has led to many new products routinely used in our day to day lives. The simplest definition of biotechnology is “applied biology”, which means the use of scientific techniques and knowledge and applying it to the development of products and new technologies. Biotechnology is more commonly referred to the use of living organisms or active molecules to make new products or control processes, such as fermentation.

Bacteria are the most frequently used living organisms in biotechnology, where they have multiple uses. Although bacteria are often associated with infection, disease and decay, there are many “helpful” bacteria. For example, bacteria are used in the fermentation of milk to produce healthy yogurt and cheeses. In the 1970s, scientists learnt how to manipulate bacteria by inserting the genes of proteins into bacteria and essentially converting the bacteria into living factories. The bacteria would produce the protein of the inserted gene and produce large amounts of the desired protein. This technique has been used for such products as human insulin for diabetic treatment, human growth hormone and several vaccines.

Proteins are small molecules that are often referred to the building blocks of life. These are encoded by individual genes on the genome. Proteins are found in every tissue of the body and are responsible for the body’s structure, controlling the cellular processes, and every other process required for the survival of a living being.

Enzymes are specialized proteins that act as catalysts for a multitude of cellular processes. The enzymes increase the rate (speed) of a reaction, but the enzymes are not involved in the chemical reaction, i.e. they aid but are not changed themselves. Enzymes have multiple roles in the human body from the digestion of food to the synthesis of new proteins. An enzyme involved in food digestion is amylase, as enzyme found in saliva. The amylase is responsible for breaking down long chained sugars into smaller sugar molecules.

This kit allows students to visualize proteins, to witness an enzyme reaction using one of their own enzymes and grow bacteria found in their mouths.

TEACHER’S PRE EXPERIMENT SET UP

This kit contains three individual experiments and can be carried out together by utilizing the incubation times of the various experiments. Timing notes are found in the protocol.

Testing Protein Enzyme Activity in Saliva

1. Heat a waterbath or heating block at 50-80°C. Lower temperatures can be used, down to room temperature, however longer incubation times will be required.
2. Prepare the Enzyme Detection Dye: Using a large transfer pipette, add 1ml Enzyme Color Dye-I solution to the vial of Enzyme Color Dye-II (fill to 1.0 graduated mark or until half full). Mix the contents by inverting the tube until the tube contents have dissolved. This may take up to 10 minutes to completely dissolve. Transfer the dissolved Enzyme Color Dye-II into the remaining Enzyme Color Dye-I solution and briefly mix the solution. This is the Enzyme Detection Dye.



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Visualization of Bacteria



Wear heat protective gloves throughout the agar melting and pouring procedure

Make Agar plates the day before the experiment.

1. Loosen the cap of the bottle containing the agar.
2. Place the bottle in a large container, such as a beaker or saucepan and add water to the container up to the level of the agar.
3. Heat the water until it begins to boil. Simmer for 1 hour and swirl the agar bottle every 5 minutes, until all the agar has melted.
4. Turn off the heat.
5. Remove the bottle from the water bath. Allow the agar to cool to the point it can be held comfortably in your hand, this takes 20-40 minutes.
6. Using aseptic techniques (figure 1), pour a ~0.5cm/ ¼” layer of agar into each Petri dish. This is approximately 15ml.



Figure 1

7. Replace the lids. Do NOT move the plates until the agar is completely set. This takes 20-30 minutes
8. Once completely set, store the Petri dish upside down (figure 2) in a refrigerator until needed. For long term storage, wrap in a plastic bag or store in an airtight container.



Figure 2

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MATERIALS FOR EACH GROUP

Supply each group with the following components. Some of the components are shared by the whole class and should be kept on a communal table.

Visualization of Proteins in Saliva

1 bottle Protein Dye Solution with large transfer pipette (shared with class)
15 Centrifuge Tubes (2ml)
5 Small Transfer Pipettes
Water
Marker Pens

Testing Enzyme Activity in Saliva

1 bottle Enzyme Detection Dye with large transfer pipette (shared with class)
10 Centrifuge Tubes (2ml)
1 bottle Enzyme Substrate (starch) with large transfer pipette (shared with class)
5 Small Transfer Pipettes

Visualization of Bacteria

1 LB Agar Petri Dish
5 Inoculating Loops

PROCEDURE

Visualization of Proteins in Saliva

This experiment uses a color dye that binds to proteins and produces distinctive blue color and enables the visualization of protein.

1. Write your name on a Centrifuge Tube (2ml). Collect your saliva by spitting a small amount, approximately a fifth of the tube volume, into the clean 2ml tube provided. Tap the tube to get the saliva to the bottom.



When collecting the saliva, it is important that the majority of the saliva is liquid as opposed to bubbles. This saliva will be used for visualization of proteins AND the enzyme experiment (see later).

2. Label two clean Centrifuge Tubes with either “Control” or “Saliva”.
3. With a large transfer pipette add Protein Dye Solution to the “Control” and “Saliva” tubes, filling to the 1.0 mark.
4. Using a transfer pipette, add one drop of water to the “Control” tube.
5. Using the same transfer pipette, add one drop of your saliva (from Step 1) to the “Saliva” tube.
6. Close the tubes and invert the tube 5-6 times to mix.
7. Let the tubes stand in a tube rack for 5 minutes. Make note of color changes.

Testing Enzyme Activity in Saliva

From the protein experiment above, we know that saliva contains proteins. Some of these proteins are specialized proteins, known as enzymes. One of these enzymes is a digestive enzyme required to start breaking down food as you chew; Amylase, the enzyme, breaks down food starch (found in wheat, rice, potatoes and other food materials) in the mouth. This experiment will allow you to visualize the reaction of the enzyme in your saliva.

1. Label two clean Centrifuge Tubes with either “Control” or “Saliva”.



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2. With a large transfer pipette fill the tubes with Enzyme Substrate (starch solution) to the 0.5 mark of each tube.
3. Using a small, clean transfer pipette, add one drop of water to the “Control” tube.
4. Using the same transfer pipette, add one drop of your saliva (from Step 1) to the “Saliva” tube.
5. Close the tubes and invert the tube 5-6 times to mix.
6. Allow the enzyme to work by leaving in a tube rack for 15-20 minutes.
7. At the end of 15-20 minutes, open tubes and add Enzyme Detection Dye to both tubes with a large transfer pipette, filling to the 1.5 mark. Mix the contents by inverting the tubes 5-6 times.
8. Carefully place both tubes in the warm water bath (50-80°C) for 15-20 minutes, or until a color change occurs.



WARNING: Be careful of the hot water, it may burn.

NOTE: Depending on the temperature of the bath or incubator the reaction may proceed too rapidly making it difficult to see a difference between the tubes. Check the tubes every 3-5 minutes and note the color differences.

9. Remove the tube from the hot water bath after 15-20 minutes and stand in the tube rack for observation.

Visualization of Bacteria

Bacteria are found almost everywhere, including in your own mouth. Bacteria, often associated with disease and illness, are very helpful to living organisms; in fact all animals would struggle to survive without them. Bacteria in our mouth perform such tasks as eating each other, including harmful bacteria, fending off bad species of bacteria, manufacturing different products, and eating the food that becomes lodged in parts of our mouths. In Biotechnology, bacteria are used for multiple applications, including as small living factories. This experiment will allow you to grow your “own” bacteria and visualize them.

1. Keeping the lid in place, turn the Petri dish upside down. Divide the bottom of the Petri dish into six even sections with a marker pen.
2. Next, each student writes his or her name in a different section and in the sixth section write “Control.”
3. Each student takes an inoculating loop, examines the loop packet and opens it at the end opposite the loop.
4. Hold the loop in one hand and place the loop end of the inoculating loop into your mouth and collect a sample from the inside of your mouth. Scrape the inside of your cheeks, your tongue and under your tongue.



Caution: Do not put the loop too far into your mouth.

5. With your free hand, remove the lid of the Petri dish and hold in your hand; to prevent contamination, do not place the lid on a lab surface. Be careful not to touch the agar or breathe on the Petri dish. Remember, bacteria are everywhere.
6. To transfer the bacteria from your mouth to the plate, carefully, gently and quickly drag the loop over the soft agar surface in zigzag manner (see figure 1). Replace the lid and pass the plate to the next student. Each student spreads their own sample in their section marked with their name. Nothing is placed in the “Control” section.

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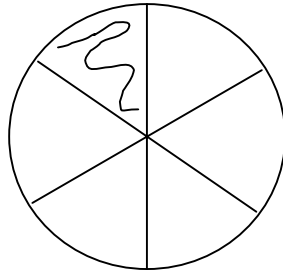


Figure 1

- Place the lid on the plate and place the Petri dish upside down in a 37°C incubator. If an incubator is not available leave at room temperature.
- Observe Petri dish 24-48 hours later.

RESULTS, ANALYSIS & ASSESSMENT

Visualization of Proteins in Saliva

Describe the difference in color between the “Control” and “Saliva” tubes:

The control tube remained a red brown color; the Saliva tube turned a blue color

Why is the tube with water called the control?

There is no protein present allowing samples with protein to be compared to a negative sample

What happens to the protein dye when it interacts with protein?

The protein dye binds the protein causing a change that results in a color change.

Is there a difference between the “Saliva” tubes of other students in the group? If so, what are the differences and what does this tell you?

The intensity of the blue color may vary and this is a reflection of the amount of protein added. The darker the blue, the more protein.

Testing Enzyme Activity in Saliva

Describe the difference in color between the “Control” and “Saliva” tubes:

The control sample remains a yellow/orange color and the Saliva tube changes to a red/ brown color.

Why do you think the dye changes color in the presence of the enzyme?

The product of the enzyme reaction interacts with the dye causing a change in its color.

Visualization of Bacteria

Write a brief description of the plate, comparing each section:

What did you expect to see on the control?

There should be no bacteria on the control

Did anything grow on the control? If so, why?

If no, then good. If yes, there was some contamination, possibly from poor aseptic techniques, allowing air born bacteria to settle on the agar or breathing on the plate or from not sterilizing the loop in the ethanol thoroughly.

Is there a difference between each section? Why do you think this is?

There are some differences, due to different levels of bacteria in student mouths and the amount applied to the agar plate.

