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The rAmylase Project by Ellyn Daugherty

Assaying for Amylase Activity

(Lab 6c)

(Cat. # BTNM-6C)



Developed in partnership with

The rAmylase Project by Ellyn Daugherty

Assaying for Amylase Activity (Lab 6c)

Teacher's Guide

The following laboratory activity is adapted from "Laboratory 6c: Assaying for Amylase Activity" from *Biotechnology: Laboratory Manual* by Ellyn Daugherty. For more information about the program, please visit www.emcp.com/biotechnology.

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About Ellyn Daugherty: Ellyn Daugherty is a veteran biotechnology educator and recipient of the Biotechnology Institute's National Biotechnology Teacher-Leader Award. She is the founder of the San Mateo Biotechnology Career Pathway (SMBCP). Started in 1993, SMBCP has instructed more than 7,000 high school and adult students. Annually, 30-40 SMBCP students complete internships with mentors at local biotechnology facilities.

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About G-Biosciences: In addition to the Biotechnology by Ellyn Daugherty laboratory kit line and recognizing the significance and challenges of life sciences education, G-Biosciences has initiated the BioScience Excellence™ program. The program features hands-on teaching kits based on inquiry and curiosity that explore the fundamentals of life sciences and relate the techniques to the real world around us. The BioScience Excellence™ teaching tools will capture the imagination of young minds and deepen their understanding of various principles and techniques in biotechnology and improve their understanding of various social and ethical issues.

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The rAmylase Project by Ellyn Daugherty

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Upon receipt, store the materials as directed in the package literature.

MATERIALS INCLUDED

This kit has enough materials and reagents for 8 lab groups (32 students in groups of 4).

- 8 tubes of 0.5mg/ml α-Amylase from *Bacillus subtilis* (2ml)
- 1 bottle 2% Starch Solution (200ml)
- 8 tubes of 1X PBS, pH 7.0 (2ml)
- 8 tubes of Lugol's Iodine Starch Indicator Solution (0.2ml)
- 8 tubes of Benedict's Solution (3ml)
- Eight 24-well Microtiter Plate
- 100 Glucose Test Strips
- Microcentrifuge Tubes (2 bags of 50)
- Lid Locks (24)

ADDITIONAL EQUIPMENT & MATERIALS REQUIRED

The following standard lab equipment should be available for each group.

- Heat block (preferred) or water bath set at 90°C
- Micropipets and tips
- 8 Small paper cups

SPECIAL HANDLING INSTRUCTIONS

- Store the Amylase frozen at -20°C.
- All other reagents can be stored at room temperature

GENERAL SAFETY PRECAUTIONS

- The reagents and components supplied in the *The rAmylase Project*™ kits are considered non-toxic and are safe to handle (unless otherwise noted), however good laboratory procedures should be used at all times. This includes wearing lab coats, gloves and safety goggles.
- The teacher should 1) be familiar with safety practices and regulations in his/her school (district and state) and 2) know what needs to be treated as hazardous waste and how to properly dispose of non-hazardous chemicals or biological material.
- Students should know where all emergency equipment (safety shower, eyewash station, fire extinguisher, fire blanket, first aid kit etc.) is located and be versed in general lab safety.
- Remind students to read all instructions including Safety Data Sheets (SDSs) before starting the lab activities. A link for SDSs for chemicals in this kit is posted at www.gbiosciences.com
- At the end of the lab**,** all laboratory bench tops should be wiped down with a 10% bleach solution or disinfectant to ensure cleanliness.
- Remind students to wash their hands thoroughly with soap and water before leaving the laboratory.

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TEACHER'S PRE EXPERIMENT SET UP

1. Label 8 beakers "2% Starch." Invert the bottle of 2% starch to ensure all of the starch is suspended. Aliquot 20ml in to each labeled beaker.

2. Distribute the following items to each lab group:

    - 1 tube of 0.5mg/ml α-Amylase from *Bacillus subtilis* (2ml)
    - 1 beaker of 2% Starch Solution (20ml)
    - 1 tube of 1X PBS, pH 7.0 (2ml)
    - 1 tube of Lugol's Iodine Starch Indicator Solution (2ml)
    - 1 tube of Benedict's Solution (4ml)
    - One 24-well Microtiter Plate
    - 10 Glucose Test Strips
    - 10 Microcentrifuge Tubes

TIME REQUIRED

- 30 minutes, pre-lab preparation
- Two 1 hour lab periods
- 30 minutes, post lab analysis

NEXT GENERATION SCIENCE STANDARDS ADDRESSED

- HS-LS1: From Molecules to Organisms: Structures and Processes
- LS1.A: Structure and Function

For more information about Next Generation Science Standards, visit: http://www.nextgenscience.org/

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EXPECTED RESULTS

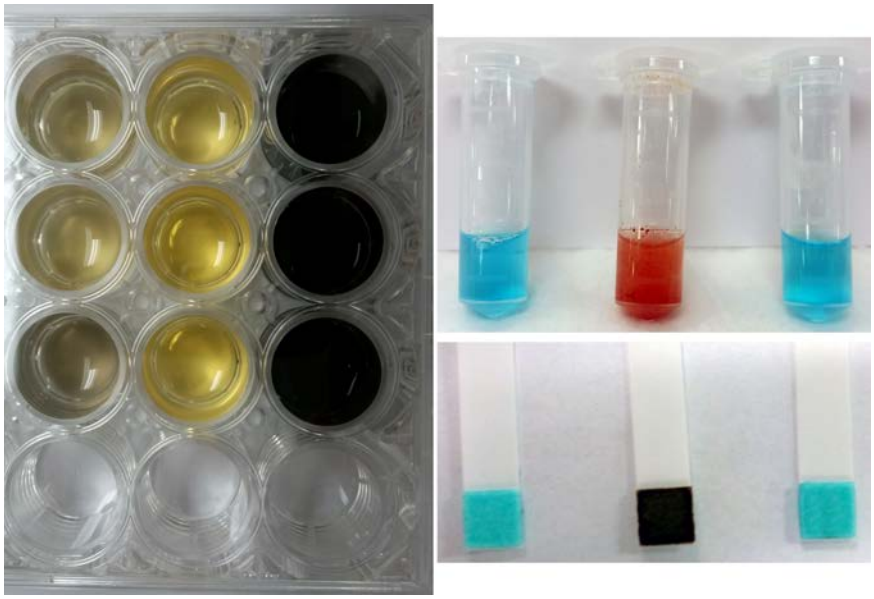


Figure: The left panel shows the starch breakdown as indicated by the Lugol's Iodine Starch Indicator Solution. Column 1 is human saliva*, column 2 is bacterial amylase and column 3 is the control. The top right panel shows the amount of sugar produced as indicated by Benedict's solution. Tube 1 is human saliva*, tube 2 is the bacterial amylase and tube 3 is the control. The bottom right panel shows the production of glucose as measured with glucose dipsticks. The left and right results have 0mg/dl glucose and are the human saliva* and control, the middle test has >2000mg/dL glucose and is the bacterial amylase. The human saliva results will vary due to differing concentrations of amylase in the saliva; the result will range from the PBS control to greater than the bacterial amylase results.

ANSWERS TO ADDITIONAL QUESTIONS

1. Which of the three assays give the "best" (most reliable) results? What might be a reason for this?
   Answer: The starch breakdown assay should progress for quickly and, thus, the iodine starch assay should show dramatic differences. Maltose and glucose production must be fairly high to show with the indicator strips or Benedict's.

2. If an aldose assay (Benedict's or sugar strips) shows a 100 mg/dL glucose concentration, what is that value measured in percent (%) of glucose? Show the calculations.
   Answer: If an assay shows an 100 mg/dL glucose concentration, then
   100 mg/dL x 1 dL/10mL = 10 mg/mL = 0.01 g/mL x 100 parts = 1% glucose

Why would the use of a multichannel pipette give "better" results when conducting these assays?
Answer: The use of a multichannel pipet gives better results for each measurement, and dispensing would be the same and more comparable than it would be using a traditional pipet.

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OBJECTIVES

What is the behavior of the human enzyme, salivary amylase, compared with a 0.5mg/ml bacterial amylase solution?

BACKGROUND

Amylase is an enzyme that catalyzes starch digestion (see Figure). It is used commercially in two ways: 1) to eliminate starch in products; 2) to produce sugar from starch. Using amylase to remove starch is a cheap and effective method, but substantial quantities of amylase must be produced if it is to be used commercially. Similarly, amylase is an economical way to obtain sugar for use in beverages and baked goods since sources of starch, for example, cornstarch, are more readily available than sources of sugar (sugar cane).

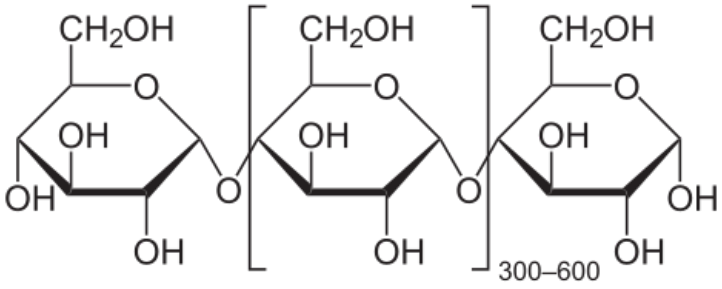


Figure: Molecular Structure of Starch. Amylose is one type of plant starch. The amylose molecule is very long, composed of hundreds of glucose molecules linked together. Amylase breaks the bond between glucose molecules in the chain to produce the disaccharide, maltose.

Some bacteria and fungi cells in nature make amylase. Several herbivorous mammals synthesize amylase as well. In humans, amylase is made in two organs involved in food breakdown. In the mouth, salivary glands produce and excrete amylase (salivary amylase) to break down the starch in food into smaller units (maltose). The pancreas is another organ that makes amylase. Amylase is produced in the pancreas (pancreatic amylase) and excreted to the small intestines where it breaks down starch to maltose. The equation for the reaction catalyzed by amylase is as follows:

$$Starch \xrightarrow{amylase} maltose + maltose + maltose$$

How might a biotechnologist know that this reaction is taking place? What assay can be used to test for the activity of this enzyme?

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MATERIALS FOR EACH GROUP

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

- Small paper cup
- 1 tube α-Amylase from *Bacillus subtilis* (0.5mg/ml) (2ml)
- 1 tube of 1X PBS, pH 7.0 (2ml)
- 2% Starch Solution (20ml)
- 1 tube of Starch Indicator Solution (Lugol's Iodine Solution) (2ml)
- 1 tube of Benedict's Solution (4ml)
- 24-well Microtiter Plate
- 12 Glucose Test Strips
- 9 Microcentrifuge Tubes (1.5ml)

Several components will be shared by the whole class and should be kept on a communal table.

Caution: Never use latex gloves when using a heat block or water bath. A 90° heat block or water bath can cause severe burns. Use lid locks to keep tubes closed. Lid locks have handles to make for safer handling. If using a hot water bath, place tubes in a floating rack so the students do not come in contact with the near boiling water.

- Lid Locks
- Heat block (preferred) or water bath set at 90°C

PROCEDURE

Enzyme Assay Set Up

One 24-well plate will be used for each lab group

1. Collect approximately 2-3ml of saliva in a clean paper cup.
   NOTE: *Human α-amylase is found in saliva. Wait 20 minutes after eating or drinking before collecting saliva. Chewing on a rubber band may increase saliva production. When collecting saliva, remember that it is a biohazard that should be treated in a mature, safe fashion. Discard the paper cup into a biohazard bag after use.*.

2. Place 1ml of starch solution in each well of the first three rows of six wells (see figure 1). The bottom row will not be used.

3. Add 300µl of human salivary amylase solution from Step 1, to the first and fourth wells of each row (see figure 1). Mix thoroughly for 2 seconds using the micropipet tip. Change tips before going to Step #4.

4. Add 300µl of bacterial amylase solution to the second and fifth wells of each row (see figure 1). Mix thoroughly for 2 seconds using the micropipet tip. Change tips before going to Step #5.

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5. Add 300µl of 1X PBS, pH 7.0 to the third and sixth wells of each row (see figure 1). Mix thoroughly for 2 seconds using the micropipet tip.

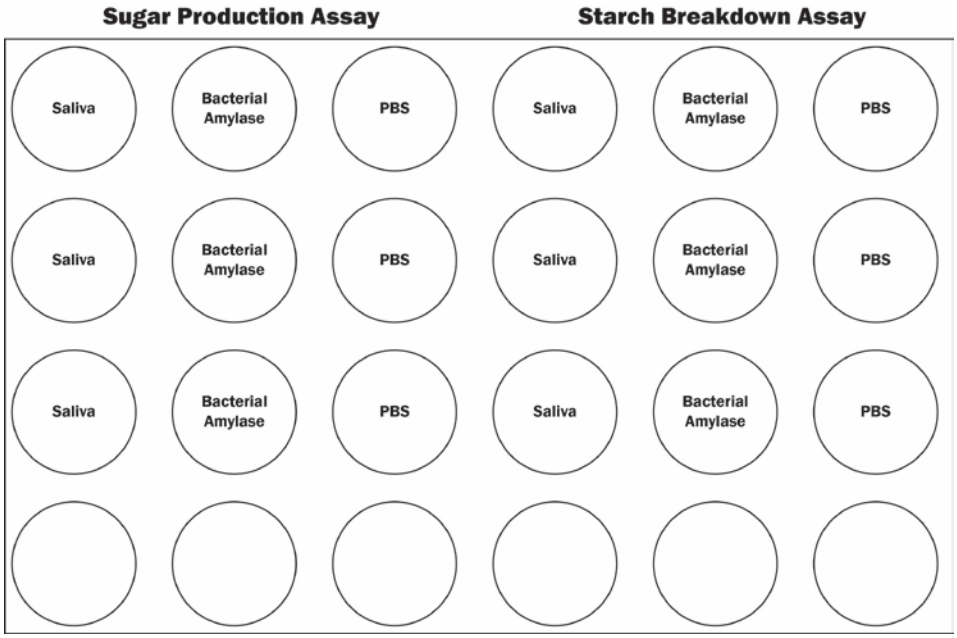


Figure 1: Set up of the Enzyme Assay Plate.

Starch Breakdown Assay

6. Add 20µl Starch Indicator Solution (Lugol's Iodine Solution) to all the fourth, fifth and sixth wells in each row (see figure 1). Mix thoroughly for 2 seconds using the micropipet tip. Change tips after each use.

7. Identify the positive and negative controls in this experiment. Make and record predictions as to what may occur in each well, including the expected color change.

8. Place the 24-well plate on a piece of white paper, out of direct light as iodine decolorizes in light. Observe until a noticeable color change occurs in the wells.

9. While waiting for a color change, create a data table to record sample data for the relative amount of starch breakdown in each well of Columns 4, 5, and 6. Include a row for average results. Starch mixed with iodine gives a very dark blue-black color. Iodine is a golden red-brown when no starch is present. Starch breakdown is measured by a decrease in the starch-iodine reaction (a lessening of the blue-black color). Make predictions about what you expect to happen in each well. What color(s) do you expect to see?

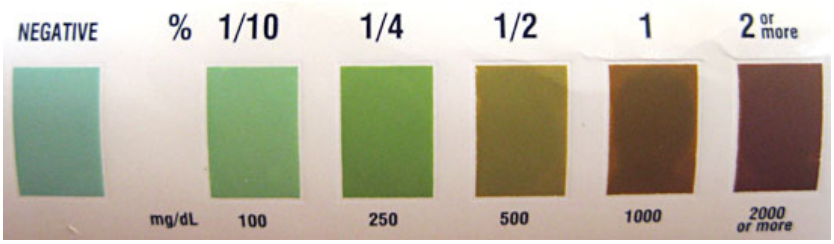
10. Measure the amount of starch breakdown (5 → 0 rating) by recording the degree of lightening of the iodine solution from black (0) to a light red-brown or clear color (5) in the wells of Columns 4, 5, and 6.

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Sugar Production Assay (Sugar Test strips)

11. Measure aldose concentration in Columns 1, 2, and 3 using supplied sugar (glucose) test strips.
12. Handling the test strip by the plastic end and dip the test indicator end into the sample. Immediately withdraw drawing the edge of the strip against the rim of the well to remove excess sample.
13. Compare the test strip to the color chart below and record the results in mg/dL.



14. Study the Sugar Test Strip color key for the units of measurement of the sugar detected by the test strips. Include data for each well in Columns 1, 2, and 3 and a row for average data. What color(s) do you expect to see after sugar strip testing?
    Although readings can be made in percent (%) or milligrams per deciliter (mg/dl), for this activity, record the data in mg/dl. A deciliter (dl) is equal to 0.1L. The mg/dl is a common unit of glucose concentration.

Sugar Production Assay (Benedict's Solution Test)

15. Cover the plate and leave it for 0.5 to 24 hours. Sugar production will be measured using both Benedict's solution and sugar test strips following the steps below.
16. Conduct the Benedict's solution test on a sample of reacted solutions from each of the wells of Columns 1, 2, and 3.
17. Label the 9 tubes to clearly identify which well they represent and add 300µl of Benedict's solution to each tube.
18. Next transfer 300µl of each sample to the appropriate tube, making sure you mix the sample with the pipette tip before removing form the well.
19. Place a locking cap on each tube and heat in a 100°C heat block for 5 minutes. Record the color change and relative amount of aldose present in each sample after heating.
    Caution: Never use latex gloves when using a heat block or water bath. A 90° heat block or water bath can cause severe burns. Use lid locks to keep tubes closed. Lid locks have handles to make for safer handling. If using a hot water bath, place tubes in a floating rack so the students do not come in contact with the near boiling water.
20. **Analysis**: Create data tables to report the relative amount of aldose present in a sample after Benedict's testing using a numerical rating system such as 5 (red-orange = high sugar) → 4 (orange) → 3 (yellow) → 1 (green) → blue (0 = no sugar) rating. Include data for each well in Columns 1, 2, and 3 and a row for average data. Make predictions about what you expect to happen in each well. What color(s) do you expect to see after Benedict's testing?

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DATA ANALYSIS & CONCLUSION

In a written concluding statement (1-3 paragraphs), discuss the results of the experiment, including your observations of the behavior of the human salivary amylase as compared to the behavior of the bacterial amylase solution. Do the data collected support your hypothetical predictions? Why or why not? How might the different enzymes (bacterial versus human amylase) react differently with the starch substrate? Discuss possible errors in experimentation that could lead to erroneous or misleading results. What variables are hard to control in this experimental design? Of what value are these types of assays? Where in industry might they be used? If you were a technician in a lab evaluating various amylase samples, what further experimentation would you suggest?

ADDITIONAL QUESTIONS

1. Which of the three assays give the "best" (most reliable) results? What might be a reason for this?
2. If an aldose assay (Benedict's or sugar strips) shows a 100-mg/dL glucose concentration, what is that value measured in percent (%) of glucose? Show the calculations.
3. Why would the use of a multichannel pipette give "better" results when conducting these assays?

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