

APPENDIX B

Links to videos used in this lab

The following videos have been included to help students understand this lab exercise, especially for online delivery.

1. **Introduction to enzymes' role in biochemical reactions:** a review of proteins/enzymes, the amylase enzyme, and the reaction it catalyzes.
<https://www.youtube.com/watch?v=V6wHXtO9kIA>
2. **Maltose standard curve:** constructing and using this graph will allow you to determine amylase activity.
<https://youtu.be/KNMz0pSgYbk>
3. **Testing your hypothesis on amylase activity:** review of the scientific method, the experimental design, and data analysis to determine the effect of an environmental factor on amylase activity.
[https://youtu.be/ BacfzIP64](https://youtu.be/BacifzIP64)
4. **Using micropipettes:**
https://youtu.be/uEy_NGDfo_8
5. **How to use a spectrophotometer:**
<https://youtu.be/u3C6QXD8gjQ>

APPENDIX C

Materials and Methods

Materials:

1. **Fungal Amylase:** *α -Amylase from *Aspergillus oryzae* (Sigma Cat# A9857-1MU)*

Stock solution (1%): Dissolve 0.5 g of BSA and 0.5 g of amylase powder to final volume of 50 ml deionized water.

Working solution (0.005%): Mix 1.0 ml of Stock solution of α -amylase from *Aspergillus oryzae* with 200 ml of deionized water containing 0.2 g of BSA. Distribute 1.4 ml aliquot of working solution to each microfuge tube and freeze.

2. **Bacterial Amylase:** *α -Amylase from *Bacillus licheniformis* (Termamyl): (Carolina Biologicals Cat# 202410)*

Stock solution (0.1%): Mix 0.25 ml of Termamyl with 250 ml of deionized water. Distribute 1.4 ml of 0.1% Termamyl to each microfuge tube and store at 4°C refrigerator.

Working solution (1:10 dilution of Stock solution): 100 ul of stock solution mixed with 900 ul of deionized water (Final dilution is 1:10000)

3. **Human Amylase:** *α -Amylase from Human saliva: (Sigma Cat# A0521-500UN)*

Stock solution: Suspend the whole powder (3.13 mg solid) in vial with 1 ml of 1% BSA.

Working solution: Dilute the working solution 1:20 times with 0.1% BSA.

5% Starch solution in deionized water: Dissolve 100g Soluble starch [from refrigerator, Cat# 85642-100G (Sigma)] in 2000 ml of deionized water and distribute to 15 ml conical tubes (14 ml in each). Store in refrigerator (4°C) for immediate use or in freezer (-20°C) for later use.

4. **0.25% Maltose in deionized water:** Dissolve 1.25g Maltose in 500 ml of deionized water and distribute in 15 ml conical tubes (5 ml in each). Store in refrigerator. Equilibrate it to room temperature before use.
5. **DNS reagent:** This reagent is a 1:1 mixture of 30% potassium sodium tartrate in 0.4 M NaOH, and 2% dinitrosalicylic acid in 0.4 M NaOH. For 2 liters DNS solution, mix 20g di-nitro-salicylic acid in 1500 ml deionized water, add slowly 300 g of Potassium sodium tartrate under constant stirring, add 64 ml of 50% W/W Sodium hydroxide (12.5 M NaOH). Finally adjust the volume up to 2 liters with deionized water.
6. **pH buffers:** pH 1 buffer (VWR cat# BDH5002-500 ml), pH 3 buffer (VWR cat# BDH5014-500 ml), pH 5 buffer (VWR cat# BDH5034-500 ml), pH 7 buffer (VWR cat# BDH5052-500 ml), pH 9 buffer (VWR cat# BDH5062-500 ml), and pH 12 buffer (VWR cat# BDH5086-500 ml).

Equipment and Materials required:

Standard curve for Maltose:

1. Maltose solution (0.25% in deionized water)
2. Deionized water
3. Six test tubes
4. DNS reagent
5. Micropipettors with appropriate tips.
6. 10 mL glass pipette and plastic pipettor
7. Spectrophotometer and a pair of cuvettes.

Hydrolysis of Starch:

1. Spectrophotometer and a pair of cuvettes.
2. Starch solution (5% in deionized water)
3. Frozen amylase (enzyme) solution in microfuge tube(s) will be given to each group. Gently thaw tubes. Do not put them in boiling water baths.
4. Water baths fixed at 37°C, 45°C, 65°C, and 85°C
5. Ice and a beaker to make a 3°C ice bath
6. Hot plate and a glass beaker for a boiling water bath
7. Twelve test tubes
8. DNS reagent
9. Micropipettors with appropriate tips for each (1000µl, 100µl).
10. 10 mL glass pipette and plastic pipettor
11. Deionized water