**Experiment X: Proteolytic Enzymes in Laundry Detergent (Tide Pods)**

**INTRODUCTION**

Tide® Pods , released in March of 2012, were introduced as “a product that will put a spark back in the laundry process” by Alexandra Keith, Vice President of Proctor & Gamble Fabric Care North America. Sparks aside, the Pods are designed to deliver a discreet amount of laundry detergent for each load in the washing machine. The Pod design features three colored compartments encased in a water-dissolvable film; while the compartments were likely implemented for purely aesthetic reasons, the Tide® Pods press release state the compartmented detergents “brighten, fight stains, and clean,” suggesting that different chemical components are present in each chamber [1].

Laundry detergents, whether liquid or solid, contain enzymes as a stain-fighting component: for amino acid-based stains – proteases; for lipid-based stains – lipases; for carbohydrate-based stains – amylases [2]. It could be inferred that these enzymes are present in one compartment of the Pods. Enzymes could even be present in two or all of the compartments. The question this procedure seeks to answer is, which one(s)?

The enzymatic activity of proteases can be measured via enzyme kinetics. For the first part of the experiment, detergent will be extracted from each chamber of a Tide® Pod and introduced into a solution containing protamine. Samples will be taken from these solutions at 2, 20, and 60 minutes after the beginning of the reaction.

When detergent from the compartment containing protease is combined with a protein substrate, the substrate should be broken down into smaller pieces over time. This will be reflected in electrophoresis as protein bands congregated closer to the positive end (bottom) of the gel. The compartment(s) containing protease should have more of these smaller-protein bands compared to enzyme-free compartment(s) and/or the control.

[1] Proctor & Gamble, . "Tide® Puts a Spin on Laundry with the Introduction of Tide® Pods™ Breakthrough technology changes laundry for good: the cleaning power of Tide®, now in the palm of your hand." P&G Corporate Newsroom. 6 3 2012: n. page. Web. 19 Sep. 2012. <http://news.pg.com/press-release/pg-corporate-announcements/tide-puts-spin-laundry-introduction-tide-pods>.

[2]  [www.spolem.co.uk: Industrial uses of enzymes](http://www.spolem.co.uk/worksheets/docs/industrial_enzymes.doc)

**EXPERIMENTAL**

|  |  |
| --- | --- |
| **Equipment and Supplies** | **Biochemicals and Related Materials** |
| Day 1: | Day 1: |
| Microcentrifuge tubes | Tide Pod |
| Micropipettes and tips | Protamine substrate solution (2.5 mg/mL) |
| Microcentrifuge | Distilled water |
| Sharpie marker | Deionized water |
| 3 – 1 mL syringe | Electrophoresis sample buffer 2x |
|  | 0.25 M Tris-HCl pH 7.5 |
|  |  |
| Day 2: | Day 2: |
| AU-PAGE gel casting apparatus | 9 enzymatic reactions (from Day 1) |
| BioRad Power supply | 1 control (from Day 1) |
| BioRad Dummy gel | Protein ladder |
| Gel loading tips | Coomassie stain solution |
| Gel comb | Destain solution |
| BioRad gel electrophoresis chamber | Running buffer (5% acetic acid) |
| Microcentrifuge tube rack | 15% acrylamide gel  |
| Balance | - 6 mL 30% acrylamide/0.2% bisacrylamide |
| Weighing boats/paper | - 1.5 mL 43% acetic acid |
| Microspatula | - 3 mL 10 M urea |
| 15 mL Falcon tube | - 1.5 mL water |
| Sharpie marker | - 10.5 mg thiourea |
|  | - 67.5 µL 30% hydrogen peroxide |
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**PROCEDURE**

*You will work with a partner for this experiment.*

**Day 1:**

Part A: Preparation of the enzymatic extract

*(Materials: 3 microcentrifuge tubes, 1 mL syringe, 3 needles, p1000 pipette and tips, dH2O, centrifuge)*

1. Using the 1 mL syringe, extract **~500 mL** from each section of the Tide Pod, place each in a separate clearly labeled 1.5 mL microcentrifuge tube.
2. Add **500 μL of water** to each tube to dissolve any particles (use the vortex).
3. Centrifuge tubes for 3 min at 10,000 rpm in the microcentrifuge to remove any insoluble particles.
4. **Reserve the supernatant** to be used for the protein digestion.

Part B: Preparing the digestion reaction

*(Materials: 3 microcentrifuge tubes, p100 & 1000 pipettes and tips,* ***deionized water****)*

1. Mix:
	1. 600 μL of the protein substrate solution [protamine (2.5 mg/ml in water)].
	2. 300 μL of **deionized water**,
	3. 105 μL of the buffer (10x,0.25 M Tris-HCl) and finally
	4. 45 μL of the supernatant (Step 4).
2. Incubate at room temperature.

Part C: Stopping the reaction

*(Materials: 9 microcentrifuge tubes, p1000 pipette and tips)*

1. Label 9 microcentrifuge tubes: Wht, Blu, Or at 2, 20, and 60 min.
2. Pipet 350 μL of 2x electrophoresis sample buffer into each tube.
3. At each of the selected times, remove 350 μL of the digestion into each appropriately labeled tube.

Part D: Preparing the Control

*(Materials: 1 microcentrifuge tube, p100 & 1000 pipettes and tips,* ***deionized water****)*

1. Mix:
	1. 200 μL of the substrate protein solution
	2. 115 μL of deionized water
	3. 35 μL of the buffer (10x,0.25 M Tris-HCl) and
	4. 350 μL of 2x electrophoresis sample buffer.

**Day 2:**

Part A: Setting up the casting tray

1. Align the glass mold and cover so that they are flush at the top, bottom, and edges.
2. Set the glass mold combination onto the rubber base of the gel casting apparatus. Clip into place using the clip at the top of the apparatus. Push green tabs backwards to lock glass plates together.
3. Set a 15 well comb nearby for insertion after gel mixture is added to the mold.

Part B: Prepare 15% acrylamide gel

1. To make a 15% acrylamide gel, add :
	1. 6 mL 30% acrylamide/0.2% bisacrylamide
	2. 1.5 mL 43% acetic acid
	3. 3 mL 10 M urea
	4. 1.5 mL deionized water
	5. 10.5 mg thiourea
2. Gently dissolve solution by multiple slow inversions. DO NOT SHAKE!

***CAREFULLY READ AND UNDERSTAND STEPS***

**3.** Once dissolved, add 67.5 µL of 30% hydroxide peroxide and invert 2-3 times.

**4.** Rapidly transfer the mixture to the casting apparatus in 1000 µL quantities using a p1000 micropipette. Should take ~6 mL of gel solution. Fill to the brim of the glass mold. **MAKE SURE THERE ARE NO BUBBLES!**

**5.** Place the comb into the appropriate slot of the gel casting mold by sliding one side in followed by the next side. *NOTE: Gel should overflow some.*

**6.** Allow the gel to solidify at room temperature for 5 – 10 min.

**7.** Carefully remove the comb from the solidified gel.

Part B: Load and run AU-PAGE gel

1. Obtain your 9 enzymatic reactions and 1 control reaction from Day 1.
2. Set up BioRad gel running apparatus.
3. Insert gel and dummy BioRad gel into apparatus.
4. Fill inner chamber with running buffer (5% acetic acid).
5. Carefully remove comb.
6. Load 15 µL of each sample into the wells. Wipe down the pipette tip with a Kimwipe before loading the solution in the well; this should minimize contamination of the running buffer with the electrophoresis sample buffer.
7. Load 10 µL of protein ladder into one of the remaining wells.
8. Record the well contents in your lab notebook.
9. Run your gel at 20 mA (constant A) for 30 min.
10. Place gel into a labeled sandwich container and rinse with distilled water. Carefully separate the glass plates using the green plastic wedge tool.
11. Rinse gel 3 times with distilled water and cover with a centimeter of stain solution.
12. After 15 minutes, replace stain solution into stock bottle.
13. Cover gel with a centimeter of destain solution.
14. After 15 minutes, discard the solution down the sink and rinse 3x with distilled water. Repeat destain procedure if necessary.
15. After destaining, rinse with distilled water.
16. Take a picture of your gel using the gel documentation system in S306.

**LABORATORY REPORT DISCUSSION QUESTIONS**

1. According to the experiment, which compartment(s) contained proteases? Did this match up with any hypotheses you made?
2. Based on the gel results, at which time point (2, 20, or 60 minutes) does most of the enzymatic reaction appear to have been completed?
3. Did you try more than one substrate and/or electrophoresis method? If so, which provided the clearest results? Speculate as to why.
4. Which parts of the experimental setup could be improved?

**Time for a bit of research:**

1. What common clothing stains are amino acid-based?
2. Most enzymes used in laundry detergents are taken from bacteria that live in hot springs. Why do you think that is?
3. Have you ever used a Tide® Pod before? What characteristics (not necessarily biochemical) might make it appealing to consumers?