Lipase Activity Lab
Adapted from Clinical Laboratory Methods, 8th Ed. By T. Gries

As we have seen in class, lipase is an enzyme that hydrolyzes fats (triacylglycerols) into freed fatty acids and glycerol. The following assay is a version of the clinical chemistry lab test for lipase activity. In the clinical setting, blood serum would be incubated with the olive oil substrate instead of the prepared lipase solutions as in this experiment. Every other action would be the same. As in the clinical setting, the purpose of the experiment is to determine the lipase activity of an unknown. Normal serum lipase values range from 0.1 to 1 unit per mL of serum. Elevated lipase activities may indicate pancreatitis or pancreatic carcinoma among other pancreatic diseases. Olive oil emulsion will be acted upon by lipase for 60 minutes at 37°C. The freed fatty acids will be neutralized by titration with NaOH. Thymolphthalein will be used as an indicator, which turns light blue at the equivalence point. To prepare for lab, make sure to calculate how to mix solution components before arriving.

Procedure
Provided materials:

200 mM Tris buffer (pH 7.7 at 37°C)
100% Olive oil emulsion
95% Ethanol
0.9% (w/v) Thymolphthalein solution
0.010 M NaOH
Porcine Intestine Extract.

1. Prepare 25 mL of a solution with the following final concentrations:
   27 mM Tris buffer
   40% (v/v) olive oil emulsion

2. Aliquot 2.167 mL of the above solution (from #1) into each of eight labeled glass test tubes (labeled *A* through *H)*.

3. Transfer 1.5 mL of the porcine intestine into a labeled 1.7 mL microfuge tube.

4. Heat the tube to 90 C for 5 minutes.

5. Add 333 uL of heated intestine extract to tubes *A, B*, *C and D*.

6. Add 333 uL of unheated intestine extract to tubes *E*, *F, G, and H*.

7. Mix each test tube by covering with parafilm and inverting multiple times.

8. Incubate tubes at 37°C for 60 minutes.

9. After 60 minutes, transfer the contents of each test tube to a clean, labeled Erlenmeyer flask. Immediately, add 1.0 mL of 95% ethanol to each flask and swirl to quench the reactions.

10. Add 4 drops of thymolphthalein indicator solution to each flask.

11. Titrate with NaOH until a light blue color appears being careful to continue mixing well.

Calculations

One unit of lipase will hydrolyze 1.0 μmole of fatty acid from a triacylglycerols in one hour at 37°C. Complete the following tables in your notebook:

Table 1: Titration results for heated porcine intestine

Tube	Volume of 0.010 M NaOH (mL)	Moles of NaOH	μmole of NaOH
B			
C			
D			
		Average (± SD):	

Table 2: Titration results for active porcine intestine

Tube	Volume of 0.010 M NaOH (mL)	Moles of NaOH	μmole of NaOH
F			
G			
H			
		Average (± SD):	

Table 3: Determination of Lipase Activity

Inactivated Average μmole of NaOH (± SD):	
Active Average μmole of NaOH (± SD):	
μmole of Fatty Acids Freed in 60 minutes (± SD):	
Total units of lipase activity (± SD):	
Units of Lipase Activity per gram of intestine (± SD):	