Lipase activity test

Purpose:

To test whether the enzymes expressed by our group are active, we designed a series of enzyme activity experiments. Lipase activity experiments are an important part of this.

Materials:

1 Lipase Activity Assay Kit, Catalog Number MAK046 (Sigma-Aldrich);
2 Pancreatic lipase-like protein (serial number 16);
3 Transparent 96-well plate

method:

Based on the instructions in the Lipase Activity Assay Kit, we designed the relevant experiments as follows:

1 Set Glycerol Standards for Colorimetric Detection: Dilute 10 µL of the 100 mM Glycerol Standard with 990 µL of the Lipase Assay Buffer to prepare a 1 mM standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Lipase Assay Buffer to each well to bring the volume to 50 µL.

2 Sample Preparation: For the rigor and scientific nature of the experiment, we divided our enzymes into three concentrations for each experiment, and each concentration was subjected to three parallel replicate experiments. The concentration gradients we set are: 1× (0.017mg/mL), 10× (0.17mg/mL), 100× (1.7mg/mL);

3 Assay Reaction

3.1 Set up the Reaction Mixes according to the scheme in Table 1. 100 µL of the Reaction Mix is required for each reaction (well). Note: Glycerol in the samples will generate a background signal. To remove the effect of glycerol background, a Sample Blank may be set up for each sample by omitting the Lipase Substrate.

Reagent	Standards and Samples	Sample Blank
Lipase Assay Buffer	93 mL	96 mL
Peroxidase Substrate	2 mL	2 mL
Enzyme Mix	2 mL	2 mL
Lipase Substrate	3 mL	–

Table 1. Reaction Mixes

3.2 Add 100 µL of the appropriate Reaction Mix to each of the wells. Mix well by pipetting.

3.3 Incubate the plate at 37℃. After 2–3 minutes ($T_{initial}$), measure the absorbance at 570 nm [$(A_{570})_{initial}$]. Note: It is essential [$(A_{570})_{initial}$] is in the linear range of the standard curve.

3.4 Continue to incubate the plate at 37℃ measuring the absorbance [$(A_{570})_{final}$] after 120 minutes. Protect the plate from light during the incubation.

4 Results:

4.1 Correct for the background by subtracting the final measurement [$(A_{570})_{final}$] obtained for the 0 (blank) glycerol standard from the final measurement [$(A_{570})_{final}$] of the standards and samples. Background values can be significant and must be subtracted from all readings. Plot the glycerol standard curve.

	Amount of substance (nmol)	0	2	4	6	8	10
Parallel repeat	Abs1	0.062	0.431	0.745	1.17	1.379	1.625
	Abs2	0.06	0.414	0.749	1.06	1.309	1.748
	Abs3	0.061	0.396	0.777	1.1	1.357	1.804
	Abs average	0.061	0.413666667	0.757	1.11	1.348333333	1.725666667
	Blank	0.052		0.053		0.051	
	Blank average	0.052					
	Abs after removing blank	0.009	0.361666667	0.705	1.058	1.296333333	1.673666667

Table 2. Glycerol standard

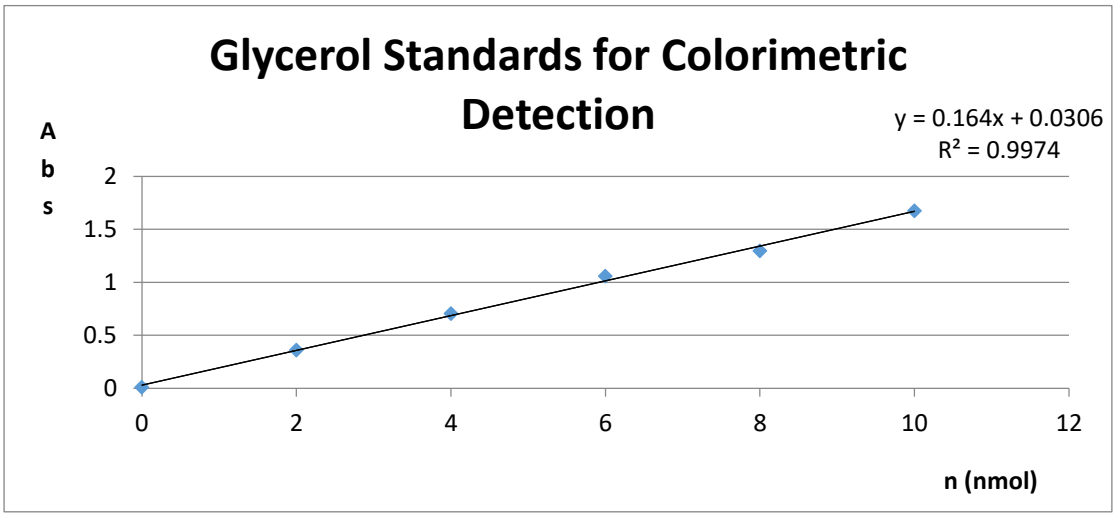

Figure 1. Glycerol standard curve

4.2 Calculate the change in absorbance from $T_{initial}$ to T_{final} for the samples.

$$\Delta A_{570} = \Delta(A_{570})_{final} - \Delta(A_{570})_{initial}$$

4.3 The lipase activity of a sample may be determined by the following equation:

$$\text{Lipase Activity} = \frac{B \times \text{Sample Dilution Factor}}{}$$

(Reaction Time) × V

B = Amount (nmole) of glycerol generated between $T_{initial}$ and T_{final}.

Reaction Time = $T_{final} - T_{initial}$ (minutes)

V = sample volume (mL) added to well

4.4 Lipase activity is reported as nmole/min/mL = milliunit/mL. One unit of Lipase is the amount of enzyme that will generate 1.0 µmole of glycerol from triglycerides per minute at 37 ℃.

4.5 Our lipase activity is 6.88 milliunits/L