Invertase Activity Assay

This procedure applies to all products that have enzymatic activity of invertase.

$$\text{Sucrose} + H_2O \rightarrow \text{Fructose} + \text{Glucose}.$$

In this particular protocol we check the activity of invertase produced by *Bacillus subtills* (if produced and secreted). Before starting this experiment, please make sure all the procedures written under 'Before Usage' had already been done.

In this protocol our objective in to detect differences in spectrophotometry of the test tubes (derived from different concentrations of enzyme) to plot a graph. We will then use the graph's slope to determine the kinetics of the bacterial enzyme.

Please notice: Test tubes "Std blank" will contain purified water only and will be used to normalize the absorbance of the light.

Please make sure to perform all tubes in triplicate.

Materials:

- Distilled Water
- 1M sodium carbonate in volume of 20 mL
- 52.37mM Picric acid solution, in volume of 25 mL
- 29.21mM of sucrose solution, in volume of 12.5 mL
- Solutions containing increasing amounts of enzyme, containing 0.1-2 U/ml
- Solution containing secreted enzyme, in volume of 1 mL
- Solution containing lysate enzyme, in volume of 1 mL

1. 30 mL of 1M sodium carbonate:
   Add 30 mL of DW to the tube. Add 3.18 gr of sodium carbonate. Stir until fully dissolve.
2. 40 mL of 52.37mM Picric acid solution:
   Add 40 mL of distilled water and 480 mg of Picric acid powder to the tube.
   Please work in a chemical hood!
   Please notice to maintain picric acid in appropriate concentrations.
3. 4 mL of 29.21mM sucrose solution, with buffer as solvent:

Add 40 mL of water and 0.4 g of sucrose to tube. Vortex until the sucrose is no longer seen. **Do not heat!**

4. 0.35 mL of commercial enzyme solution:
   Prepare aliquot: add 10 mg of enzyme to tube. Your commercial enzyme contains 10*X U/ml, when X is the protein specific activity (mentioned by the producer).
   Dilute in proper ratio.
   Please perform this section close to the experiment!

Procedure:

1. Heat water bath to 90ºC.
2. Mix and equilibrate to 37°. Add to enzyme tubes (in mL):

	Test blank1	Secreted Enzyme Top layer	Secreted Enzyme Bottom layer	Commercial Enzyme
Water	0.4	-	-	-
Sucrose solution	0.6	0.6	0.6	0.6

Perform all samples in triplicate (i.e. make sure to prepare 3 samples of each kind)

Reagent	Secreted Enzyme Top layer	Secreted Enzyme Bottom layer	Commercial Enzyme
Secreted enzyme- top layer	0.4	-	-
Secreted enzyme-bottom layer	-	0.4	-
Commercial enzyme solution	-	-	0.4

3. Mix by swirling and incubate at 37ºC for exactly 10 minutes respectively. Swirl and invert tubes after the t-minute incubation.
4. In glass reaction tubes, add:
    - 200 µL of solution
    - 800µL of water
    - 500 µL sodium carbonate solution
5. Add 1mL of picric acid to all tubes.
6. Incubate in 90ºC bath for 10 minutes.
7. Chill in room temperature. **Please mind the heat!**
8. Add 200 µL of each tube onto the 96 plate (Please document the location of each sample carefully) and perform photo-spectrometry at 492 nm light.

Calculations

- For each kind of solution performed in triplicate, calculate the mean value.
- Corrected Absorbance: $\Delta A_{492nm} = A_{492nm_{sample}} - A_{492nm_{blank}}$
- Prepare a standard curve by plotting the ΔA492nm versus the enzymatic activity.
- Enzymatic activity $= \frac{\Delta A_{492nm}(Test))}{slope}$

Appendix- Calculations of concentrations of solutions:

1. 52.37mM Picric acid solution, in volume of 40 mL

    In case of powder

$$v_{solution} = 40mL, Mw_{picric\ acid} = 229.01\frac{gr}{mol} \rightarrow m = 40mL * 0.05237\frac{mol}{l} * 10^{-3}\frac{l}{ml} * 229.01\frac{gr}{mol} * 1000\frac{mg}{gr}$$
$$= \mathbf{480\ mg}$$

2. 1M sodium carbonate in volume of 30 mL

$$v_{solution} = 30mL, Mw_{Na_2CO_3} = 105.9888\frac{gr}{mol} \rightarrow m = 30mL * 1\frac{mol}{l} * 10^{-3}\frac{l}{ml} * 105.988\frac{gr}{mol} * 1000\frac{mg}{gr}$$
$$= \mathbf{3180\ mg \sim 3.18\ gr}$$

3. 29.21mM of sucrose solution, in volume of 45 mL

$$v_{solution} = 40mL, Mw_{Sucrose} = 342.3\frac{gr}{mol} \rightarrow m = 40mL * 0.02921\frac{mol}{l} * 10^{-3}\frac{l}{ml} * 342.3\frac{gr}{mol} * 1000\frac{mg}{gr} = \mathbf{450mg}$$