Glucose Oxidase Activity Assay

The protocol is based on the following reaction:

β -D-Glucose + O_2 + $H_2O \xrightarrow{GOX}$ D-Glucono -1,5 – Lactone + H_2O_2
H_2O_2 + 2ABTS (reduced) $\xrightarrow{HRP}$ 2ABTS(oxidized)+ $1/2O_2$ + H_2O

The reaction will use a comparison in order to detect differences in 416 nm light absorbance. The amount of glucose oxidase activity will be calculated based on the detected different in absorbance. In the first reaction, Glucose will be oxidized by GOX to create hydrogen peroxide. The hydrogen peroxide will be used as substrate in the second reaction to oxidize ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)). ABTS is an organic material soluble in water, which its oxidized form absorbs 416 nm (green) light. The ABTS will be oxidized by the **horseradish peroxidase** enzyme (HRP), which oxidizes its substrates in the presence of hydrogen peroxide (in a 1:2 ratio). Therefore, the absorbance of 416 nm light indicates us the amount of hydrogen peroxide produced, from which we can deduce the activity of GOX.

Materials:

- 23 mL of 0.1 M PB buffer
  Add 23 mL of PB to tube. Instructions for making PB can be found in "Phosphate Buffer Preparation (for GOX Activity)".
- 5.5 mL of 10 mM glucose solution
  Add 10 mg to tube.
  Add 5.5mL of DW water to tube. Vortex well until the glucose is fully dissolved in water.
- 200 µL of commercial Glucose Oxidase (GOX) solution
  Please prepare this solution close to experiment
  Prepare aliquot: Weigh 10mg of Glucose Oxidase enzyme. add 1ml of water. Your solution now contains 10*X U/ml, when X is your specific activity mentioned by the producer.
- 300 µL of horseradish peroxidase (HRP) solution
  Please prepare this solution close to experiment
  Weigh $\frac{330U}{hrp\ specific\ activity\ (\frac{U}{mg})}$ mg HRP enzyme. Add 1 ml of water to tube.
- DW water
- 1 mL of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) solution
  Weigh 3.71 mg of ABTS in Eppendorf tube and add 1000 µL of 0.1M PB buffer
- 100 µL of secreted enzyme solution.
- 100 µL of bacterial Lysate solution.
- 200µL of 2M HCl solution.
- Stop-watch

Procedure:

Note: "Commercial enzyme 1-5" tubes will be used to create a standard curve- Please prepare those tubes in duplicate. Other tubes are samples, please prepare them in triplicate.

1. Prepare samples of standard curve (in duplicate):

Reagent	Blank	Commercial enzyme 1	Commercial enzyme 1	Commercial enzyme 2	Commercial enzyme 3	Commercial enzyme 4	Commercial enzyme 5
Glucose 10 mM	25 µL						
PB Buffer	905 µL	900 µL	895 µL	892.5 µL	890 µL	987.5 µL	885µL
ABTS Solution	30 µL						
HRP Solution	10 µL						
Commercial GOx solution **Add last!**	-	5 µL	10µL	12.5 µL	15 µL	17.5 µL	20 µL

2. For each tube, add 30 µL of 2M HCl solution. Make sure to do so precisely t minutes after the enzyme was added. **Please work in a chemical hood!** **Notice:** t is the number of minutes you have found in the pre-experiment section.
3. Prepare samples of secreted GOx enzyme (in triplicate):

Reagent	Secreted enzyme top layer	Secreted enzyme bottom layer
Glucose 10 mM	25µL	
PB Buffer	895µL	
ABTS Solution	30µL	
HRP Solution	10µL	
Secreted GOx solution, top layer **Add last!**	10µL	-
Secreted GOx solution, Bacteria layer **Add last!**	-	10µL

4. For each tube, add 30 µL of 2M HCl solution. Make sure to do so precisely t minutes after the enzyme was added. **Please work in a chemical hood!** **Notice:** t is the number of minutes you have found in the pre-experiment section.
5. Add 200µL of each sample and place in suitable cuvettes.
6. Measure the absorbance in 416nm light (light path=1cm).
7. For each sample, calculate the mean value.

8. Prepare a standard curve, using the blank and commercial enzyme solutions. Make sure to calculate: $Absorbance = \Delta A_{sample} - \Delta Ablank$
9. Using the curve, deduce the normalized amount of activity:

$$Normalized\ enzyme\ amount = \frac{\Delta A_{sample} - \Delta A_{blank}}{slope}$$

Please make sure your amount fits the linear part of the curve!

$$\frac{units}{ml\ enzyme} = \frac{units}{ml\ enzyme}(purchased\ enzyme\ 1\ sample) * 100 * Normalized\ enzyme\ amount$$

Where 100 is the dilution factor of commercial enzyme 1 sample.