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Megazyme Sucrose D-Glucose Assay Kit (K-SUCGL)

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Protocol for a colorimetric assay kit to determine sucrose and D-glucose concentrations by Megazyme.

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1. Store all reagent solutions as per the instructions given in the protocol. Keep the 1.65 mL working stocks for Solution 4 and Solution 4 covered in aluminium foil at all times.

2. Thaw the frozen Solution 2 (beta-fructosidase) and 1.65 mL working stocks for Solution 4 (GOPOD reagent buffer) in a 4°C refrigerator before use.

Kit contents:

Bottle 1: Acetate buffer (20 mL, pH 4.6). Store at 4°C.
Bottle 2: Beta-fructosidase (invertase) and sodium azide preservative (5 mL). Store at 4°C.
Bottle 3: GOPOD Reagent Buffer, p-hydroxybenzoic acid and sodium azide (50 mL. pH 7.4). Store at 4°C.
Bottle 4: GOPOD Reagent Enzymes - Glucose oxidase, peroxidase and 4-aminoantipyrine. Freeze-dried powder. Store at -10°C.
Bottle 5: D-Glucose standard solution (5 mL, 1mg/mL) in benzoic acid. Store at room temperature.
Bottle 6: Control flour sample. Store at room temperature.

Apparatus:
Test tubes
Microcentrifuge tubes
Pipettes
Water bath
Spectrophotometer or 96 well plate reader

Use gloves while handling Bottle 2, Bottle 3, Solution 2, Solution 3 and Solution 4 as they contain sodium azide, which is toxic

Preparing the Reagents

1 To prepare Solution 1: Acetate Buffer, dissolve the contents of Bottle 1 (**20 mL**) to **400 mL** in distilled water.

Store at **4 °C**

2 To prepare Solution 2: Beta-fructosidase, dissolve **1 mL** of solution from Bottle 2 (**20 mL**) to **10 mL** in distilled water.

For convenience, prepare ten **1 mL** aliquots of this. Store them at **-20 °C**

3 To prepare Solution 3: GOPOD Reagent Buffer, dissolve **5 mL** of solution from Bottle 3 (**50 mL**) to **100 mL** in distilled water.

Divide Solution 3 into two portions of **20 mL** and **80 mL** .

Solution 3 must be used immediately and can't be stored.

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4 To prepare Solution 4: GOPOD Reagent, dissolve all of the contents of Bottle 4 into the **20 mL** portion of Solution 3.

For convenience, divide this into twelve aliquots of **1.65 mL** each as working stocks.

Cover completely in aluminium foil and store at **-20 °C** .

Add **1.632 mL** from the **1.65 mL** aliquots of the working stock to the **80 mL** portion of Solution 3. This is your Solution 4 (GOPOD Reagent).

Cover completely in aluminum foil and store at **4 °C** .

5 To prepare fresh Solution 4 (GOPOD Reagent) in the future from the working stock aliquots:

First prepare **80 mL** of fresh Solution 3 (GOPOD Reagent Buffer) by dissolving **4 mL** of the solution from Bottle 4 to **80 mL** in distilled water.

Add **1.632 mL** from the **1.65 mL** aliquots of the working stock to this **80 mL** of freshly prepared Solution 3

Verify that the absorbance of Solution 4 at 510 nm against a distilled water blank is <0.05,

Note: Solution 3 cannot be stored and must be prepared freshly to make a fresh batch of Solution 4 (GOPOD Reagent).

Reactions: 20m

6 100ug D-Glucose standard:

Take **0.1 mL** of the solution from Bottle 5 in a test tube.

Add **0.3 mL** of distilled water.

7 Sample:

Dilute your sample according to the directions in the kit instructions or by any factor that would bring your expected sucrose+glucose concentration to the range of 0.05 g/L to 1 g/L (as per our standardization curve).

Take **0.1 mL** of the diluted sample in a **1 mL** MCT, and add **0.1 mL** of Solution 1 (Acetate buffer). Denote as Solution A.

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Take **0.1 mL** of the diluted sample in another **1 mL** MCT, and add **0.1 mL** of Solution 2 (Beta-fructosidase). Denote as Solution B.

8 Blanks:

In a **1 mL** microcentrifuge tube (MCT), add **0.1 mL** of distilled water and add **0.1 mL** of Solution 1 (Acetate buffer). This is the blank for Solution A.

In another **1 mL** MCT, add **0.1 mL** of distilled water and add **0.1 mL** of Solution 2 (Beta-fructosidase). This is the blank for Solution B.

9 Incubate the 100 ug D-glucose standard, blanks, and samples in a water bath at **50 °C** for 20m **00:20:00** .

10 Add **1.5 mL** of Solution 4 (GOPOD Reagent) to the 100 ug D-glucose standard, blanks, and sample A and B solutions. 20m

Incubate in a water bath at **50 °C** for **00:20:00** .

Solutions that contain free D-glucose or sucrose that was inverted into D-glucose by the action of beta-fructosidase should turn red.

Absorbance Measurements

11 Pipette **0.3 mL** from each of the reaction mixtures (standard, blanks, sample A and B solutions) into the wells of a 96 well plate.

12 Measure the absorbance of the reaction mixtures at 510 nm in a 96 well plate reader.

Otherwise, a regular cuvette and spectrophotometer can be used to measure absorbance.

Calculations:

13 Define ΔA as the absorbance of Solution A of the sample against the acetate buffer blank and ΔB as the absorbance of Solution B of the sample against the beta-fructosidase blank.

Define factor to convert from absorbance to ug for 100ug of D-glucose as F.

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F = 100/absorbance of the 100ug D-glucose standard.

Define D as the factory by which the sample was diluted. For example, if 1 mL of the sample was diluted to 100mL with distilled water, D = 100.

Sucrose concentration is given by:

$(\Delta B - \Delta A) \times F \times D \times 0.0095$

See the Megazyme kit instructions for the derivation of the above formula.

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