SUCROSE/D-GLUCOSE

ASSAY PROTOCOL

K-SUCGL

08/23

(250 Assays per Kit)

INTRODUCTION:

Sucrose and D-glucose are two of the most commonly occurring sugars in plant and food products and have serious impacts on human nutrition. D-Glucose can be conveniently measured in body fluids using commercially available kits based on the glucose oxidase/peroxidase or on the hexokinase/G6PDH enzymatic procedures. However, D-glucose in plant extracts usually occurs together with maltose, maltosaccharides, starch, sucrose and/or β-linked D-glucooligosaccharides. Consequently, more stringent requirements are placed on the purity of the assay reagents. The reagents must be essentially devoid of starch degrading enzymes, sucrose degrading enzymes and β-glucosidase, as these can lead to either an overestimation or an underestimation of free D-glucose present in the extract or derived by specific enzymic degradation of glucose containing oligosaccharides or polysaccharides (e.g. barley β-glucan). This Sucrose/D-Glucose Test Kit employs high purity glucose oxidase, peroxidase and β-fructosidase (invertase) and can be used with confidence for the specific measurement of D-glucose and sucrose in plant and food extracts.

PRINCIPLE:

(1)

$$\text{D-Glucose} + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{D-gluconate} + H_2O_2$$

(2)

$$2H_2O_2 + \textit{p}\text{-hydroxybenzoic acid} + 4\ \text{aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{quinoneimine dye} + 4H_2O$$

The reactions involved are:

(3)

$$\text{Sucrose} + H_2O \xrightarrow{\beta\text{-fructosidase}} \text{D-glucose} + \text{D-fructose}$$

Free D-glucose in the sample extract is determined by conversion to a red coloured quinoneimine dye compound through the action of glucose oxidase (1) and peroxidase (2) at pH 7.4, and employing *p*-hydroxybenzoic acid and 4-aminoantipyrine.

At pH 4.6, sucrose is hydrolysed by the enzyme β-fructosidase to D-glucose and D-fructose (3). The determination of D-glucose after inversion (total D-glucose) is carried out simultaneously according to the principle outlined above. The sucrose content is calculated from the difference of the D-glucose concentrations before and after enzymatic inversion.

ACCURACY:

Standard errors of less than 5% are achieved routinely.

KITS:

Kits suitable for performing 250 assays are available from Neogen. The kits contain the full assay method plus:

Bottle 1: Buffer (20 mL, pH 4.6).
Store at 4°C. See individual label for expiry date.

Bottle 2: β-Fructosidase (invertase) solution (yeast; 5 mL) plus sodium azide as a preservative (0.02% w/v).
Store at 4°C. See individual label for expiry date.

Bottle 3: **GOPOD Reagent Buffer** (50 mL, pH 7.4),
p-hydroxybenzoic acid and sodium azide (0.09% w/v).
Store at 4°C. See individual label for expiry date.

Bottle 4: **GOPOD Reagent Enzymes**. Glucose oxidase plus peroxidase and 4-aminoantipyrine.
Freeze-dried powder.
Store below -10°C. See individual label for expiry date.

Bottle 5: D-Glucose standard solution (5 mL, 1.0 mg/mL) in 0.2% (w/v) benzoic acid.
Store sealed at room temperature. See individual label for expiry date.

Bottle 6: Control flour sample. Sucrose and D-glucose contents shown on vial label.
Store sealed at room temperature. See individual label for expiry date.

PREPARATION OF REAGENT SOLUTIONS/SUSPENSIONS:

1. Dilute the contents of **bottle 1** to 400 mL with distilled water before use. This This is **solution 1** (diluted buffer). Stable for ≥ 1 year at 4°C.

2. Dilute 1.0 mL of the contents of **bottle 2** to 10 mL with **solution 1 (**diluted buffer). This is **solution 2** (diluted β-fructosidase). In dispensing this viscous liquid, a positive displacement dispenser is recommended (however, this is not essential as the enzyme is in excess). Stable for ≥ 2 years below -10°C.

3. Dilute the contents of the **GOPOD Reagent Buffer** bottle to 1 L with distilled water (this is **Solution 3**). Use immediately.

NOTE:

1. On storage, salt crystals may form in the concentrated buffer. These must be completely dissolved when this buffer is diluted to 1 L with distilled water.
2. This buffer contains 0.09% (w/v) sodium azide. This is a poisonous chemical and should be treated accordingly.

4. Dissolve the contents of the **GOPOD Reagent Enzymes** bottle in 20 mL of **solution 3** and quantitatively transfer this to the bottle containing the remainder of **solution 3**. Cover this bottle with aluminium foil to protect the enclosed reagent from light. This is **Glucose Determination Reagent (GOPOD Reagent)**.
Stable for ≥ 1 month at 4°C or ≥ 12 months below -10°C.

If this reagent is to be stored in the frozen state, it should be divided into aliquots (e.g. 200 mL in polypropylene containers). Do not freeze/thaw more than once.

When the reagent is freshly prepared, it may be light yellow or light pink in colour. Upon storage at 4°C the **GOPOD reagent** may develop a stronger pink colour The absorbance of this solution should be less than 0.05 when read against distilled water.

5&6. Use the contents of **bottles 5** and **6** as supplied.

EQUIPMENT (RECOMMENDED):

1. Glass test tubes (round bottomed; 16 x 100 mm and 18 x 150 mm).
2. Micro-pipettors, e.g. Gilson Pipetman® (100 µL, 200 µL and 500 µL).
3. Positive displacement pipettor, e.g. Eppendorf Multipette®
   - with 5.0 mL Combitip® (to dispense 0.2 mL aliquots of **solution 2** (diluted β-fructosidase) and **solution 1** (diluted buffer).
   - with 12.5 Combitip® (to dispense 1.0 mL aliquots of bottle 1 (β-fructosidase solution)).
4. Analytical balance.

5. Spectrophotometer set at 510 nm.
6. Vortex mixer (e.g. IKA® YellowLab Test Tube Shaker TTS).
7. Thermostated water bath (set at 50°C).
8. Boiling water bath (set at 85-90°C).
9. Stop clock.
10. Whatman No. 1 (9 cm) glass fibre filter papers.

CONTROLS AND PRECAUTIONS:

1. The time of incubation with **GOPOD reagent** is not critical but should be at least 20 min.
2. Include reagent blanks and D-glucose controls (100 µg quadruplicate) with each set of determinations.

   a. The **reagent blank** consists of 0.4 mL of distilled water + 3.0 mL **GOPOD Reagent**.
   b. The D-**glucose control** consists of 0.1 mL of **bottle 5** (D-glucose standard, 1 mg/mL) + 0.3 mL of distilled water + 3.0 mL **GOPOD Reagent**.

3. Analyse an extract of the control flour (**bottle 6**) with each set of determinations.
4. With each new batch of **GOPOD Reagent**, the time for maximum colour formation with 100 µg of D-glucose standard should be checked. This is usually approx. 15 min.
5. Prior to analysis sample preparation maybe necessary dependent on sample type. Refer to the sample dilution and sample preparation sections for more information.

ASSAY PROCEDURE:

Assay for Glucose and Sucrose:

1. Add 0.2 mL of sample extract (containing D-glucose + sucrose at a concentration of 0.02-0.5 mg/mL) to the **bottom** of four 16 x 100 mm glass test tubes. Add either **solution 1** (diluted buffer) or **solution 2** (diluted β-fructosidase) to duplicate tubes as follows:

   - 0.2 mL of sample + 0.2 mL **solution 1** [D-Glucose] … This is '**A'**
   - 0.2 mL of sample + 0.2 mL **solution 2** [Sucrose + D-Glucose] …..This is '**B'**

2. Incubate all tubes, including the reagent Blanks and D-glucose controls at 50°C for 20 min.
3. Add 3.0 mL of **GOPOD Reagent** to all tubes and incubate these at 50°C for 20 min.

4. Measure all absorbances at 510 nm against the reagent blank.

Absorbances: Δ**A** = GOPOD absorbance for **A**
Δ**B** = GOPOD absorbance for **B**

CALCULATIONS:

NOTE: These calculations can be simplified by using the ***Mega-Calc***™, downloadable from where the product appears on the Megazyme website (www.megazyme.com).

D-Glucose; g/L of sample solution:

$$= \frac{\Delta\mathbf{A}}{0.2} \times F \times \frac{1}{1000} \times \frac{1000}{1000} \times \text{Dilution}$$

$$= \Delta\mathbf{A} \times F \times 0.0050 \times \text{Dilution}$$

Sucrose; g/L of sample solution:

$$= \frac{\Delta\mathbf{B} - \Delta\mathbf{A}}{0.2} \times F \times \frac{1}{1000} \times \frac{1000}{1000} \times \frac{342}{180} \times \text{Dilution}$$

$$= (\Delta\mathbf{B} - \Delta\mathbf{A}) \times F \times \text{Dilution} \times 0.0095$$

where:

Δ**A**/0.2 and Δ**B -** Δ**A** /0.2
= absorbances (510 nm) (**GOPOD Reagent**) for 0.2 mL of sample treated with **solution 1** (Δ**A**) (free D-glucose); or the absorbance of 'Δ**B -** Δ**A'** where 0.2 mL of sample is treated with **solution 2** (diluted β-fructosidase) giving Δ**B** (free D-glucose plus D-glucose from sucrose) and free D-glucose (Δ**A**) is deducted to give the sucrose content.

F = factor to convert from absorbance to µg for 100 µg of D-glucose (= 100/absorbance for 100 µg D-glucose).

1/1000 = conversion from µg to mg.

1000/1000 = conversion from mg/mL to g/L.

342/180 = conversion from µg of D-glucose (as measured) to µg of sucrose.

Dilution = dilution of the original sample solution.

When analysing solid and semi-solid samples which are weighed out for sample preparation, the result is calculated from the amount weighed:

Content of sucrose

$$= \frac{c_{sucrose}\ (g/L\ sample\ solution)}{weight_{sample}\ (g/L\ sample\ solution)} \times 100 \quad [g/100\ g]$$

SAMPLE DILUTION:

The amount of sucrose and D-glucose present in the cuvette should range between 10 µg and 100 µg. The sample solution must therefore be diluted sufficiently to yield a sugar concentration between 0.02 and 0.5 g/L.

Dilution table

Estimated amount of sucrose + glucose per litre	Dilution with water	Dilution factor
< 0.5 g	-	1
0.5 - 5.0 g	1 + 9	10
5.0 - 50 g	1 + 99	100
> 50 g	1 + 999	1000

SAMPLE PREPARATION:

NOTE: Samples containing high amounts of protein or fats may cause interference in target analyte determination and require sample clarification prior to analysis using Carrez Clarification. Carrez Clarification can be simplified using the Megazyme brand Carrez Clarification kit (**K-CARREZ**) available from Neogen. The supplied **K-CARREZ** reagents should be diluted prior to use as described on page 2 of the **K-CARREZ** assay protocol and the assay procedure for clarification followed as described on page 3. The clarified sample solution prepared using the **K-CARREZ** kit should then be analysed for D-glucose/Sucrose content as described in the assay procedure.

1. Liquid foodstuffs.

Use clear, colourless or slightly coloured solutions directly or after dilution according to the dilution table. Filter turbid solutions (Whatman GF/C glass fibre filter papers) or clarify using the Carrez Clarification kit (**K-CARREZ**). Strongly coloured solutions which are used undiluted for the assay because of their low sucrose and D-glucose concentrations must be decolourised with polyvinylpolypyrrolidone (PVPP). Beverages containing gas should be degassed under vacuum.

2. Solid foodstuffs.

Solid samples (such as cereals and flours) should be homogenised using a 0.5 mm screen. Weigh out a representative sample weight (*e.g.,* 1 g) and add to a 100 mL volumetric flask and make to the mark (*i.e.* 100 mL) with distilled water. Stir vigorously until the sample is fully dispersed or dissolved, heat to 60 °C if necessary. Filter and use the clear solution, diluted further (if required) for the assay. The weights and volumes used here are provided as a guide only and may need to be altered depending on the sample being tested.

If you require further assistance with sample preparation, or information on validated matrices please contact your Neogen representative.

Contact us for more information: neogen.com/contact

Without guarantee

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