Megazyme

www.megazyme.com

D-GLUCOSE

ASSAY PROCEDURE (GOPOD-FORMAT)

K-GLUC 10/15

(660 Assays per Kit)

INTRODUCTION:

D-Glucose can be conveniently measured in body fluids using commercially available kits based on the glucose oxidase/peroxidase or on the hexokinase/G6P-DH enzymic procedures. However, D-glucose in plant extracts usually occurs together with maltose, maltosaccharides, starch, sucrose and/or β-linked gluco-oligosaccharides. 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 D-glucose containing oligosaccharides or polysaccharides (e.g. barley β-glucan). Most commercially available D-glucose kits based on the glucose oxidase/peroxidase reaction contain reagents which are not sufficiently pure.

The Megazyme D-Glucose (glucose oxidase/peroxidase; GOPOD) Assay Kit employs high purity glucose oxidase and peroxidase and can be used with confidence for the specific measurement of D-glucose in extracts of plant materials or foods. The colour which forms is stable at room temperature for at least two hours after development.

PRINCIPLE:

The reactions involved are:

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

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

KITS:

Kits suitable for performing 660 assays (3 mL per assay) are available from Megazyme. The kits contain the full assay method plus:

Bottle 1: (x 2) **GOPOD Reagent Buffer.** Buffer (50 mL, pH 7.4), *p*-hydroxybenzoic acid and sodium azide (0.095% w/v). Stable for > 4 years at 4°C.

Bottle 2: (x 2) **GOPOD Reagent Enzymes.** Glucose oxidase plus peroxidase and 4-aminoantipyrine. Freeze-dried powder.
Stable for > 5 years at -20°C.

Bottle 3: D-Glucose standard solution (5 mL, 1.0 mg/mL) in 0.2% (w/v) benzoic acid.
Stable for > 5 years at room temperature.

PREPARATION OF REAGENT SOLUTIONS/SUSPENSIONS:

1. Dilute the contents of one of bottle 1 (GOPOD Reagent Buffer) to 1 L with distilled water. **This is Solution 1**. 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 0.095% (w/v) sodium azide. This is a poisonous chemical and should be treated accordingly.

2. Dissolve the contents of one of bottle 2 in approx. 20 mL of solution 1 and quantitatively transfer this to the bottle containing the remainder of solution 1. Cover this bottle with aluminium foil to protect the enclosed reagent from light. This is **Glucose Determination Reagent (GOPOD Reagent)**.
Stable for ~ 3 months at 2-5°C or > 12 months at -20°C.

If this reagent is to be stored in the frozen state, preferably it should be divided into aliquots. Do not freeze/thaw more than once.

When the reagent is freshly prepared it may be light yellow or light pink in colour. It will develop a stronger pink colour over 2-3 months at 4°C. The absorbance of this solution should be less than 0.05 when read against distilled water.

ASSAY CONDITIONS:

Wavelength:	510 nm
Temperature:	40-50°C
Light path:	1 cm
Read against:	Reagent Blank

ASSAY PROCEDURE:

Add 3.0 mL of GOPOD Reagent to 0.1 mL of sample solution containing D-glucose and incubate at 40-50°C for 20 min (see table on next page). Read absorbances at 510 nm against the **reagent blank** to obtain ΔA_{sample} and $\Delta A_{D\text{-glucose standard}}$.

CALCULATION:

$$\text{D-Glucose } (\mu g/0.1 \text{ mL}) = \frac{\Delta A_{Sample}}{\Delta A_{\text{D-Glucose standard (100 } \mu g)}} \times 100$$

	Reagent blank	Standard	Sample
GOPOD reagent	3.0 mL	3.0 mL	3.0 mL
D-Glucose standard	-	0.1 mL	-
sample	-	-	0.1 mL
buffer or water	0.1 mL	-	-

REFERENCES:

1. Trinder, P. Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor. (1969). *Ann. Clin. Biochem.,* **6**, 24-27.
2. Blakeney, A. B. & Matheson, N. K. Some properties of the stem and pollen starches of rice. (1984). *Starch,* **36**, 265-269.
3. McCleary, B. V. & Codd, R. Measurement of (1→3),(1→4)-β-D-glucan in barley and oats: A streamlined enzymic procedure. (1991). *J. Sci. Food Agric.,* **55**, 303.

Megazyme International Ireland,
Bray Business Park, Bray,
Co. Wicklow,
A98 YV29,
IRELAND.

Telephone: (353.1) 286 1220
Facsimile: (353.1) 286 1264
Internet: www.megazyme.com
E-Mail: info@megazyme.com