Megazyme

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GOPOD REAGENT ENZYMES

(For the preparation of GOPOD DETERMINATION REAGENT)

ASSAY PROCEDURE (GOPOD-FORMAT)

R-GLC4 02/19

(1300 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 over-estimation or an under-estimation 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 Glucose Determination Reagent (glucose oxidase/peroxidase; GOPOD) 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:

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

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

KITS:

NOTE: Preparation of Glucose Determination Reagent using the **GOPOD Reagent Enzymes** (supplied) also requires the preparation of **GOPOD Reagent Buffer** (NOT SUPPLIED); see page 2.

GOPOD Reagent Enzymes for the preparation of Glucose Determination Reagent suitable for performing 1300 assays (3 mL per assay) are available from Megazyme. The kits contain the full assay method plus:

Bottle 1: (x 4) **GOPOD Reagent Enzymes.** Glucose oxidase plus peroxidase and 4-aminoantipyrine.
Freeze-dried powder. Stable for > 5 years below -10°C.

PREPARATION OF GOPOD REAGENT BUFFER (NOT SUPPLIED):

To 160 mL of stirred, distilled water add:

Potassium dihydrogen orthophosphate (MW = 136)	27.2 g
Sodium hydroxide pellets	8.4 g
p-Hydroxybenzoic acid (MW = 138)	6 g

Stir to dissolve then adjust the pH to 7.4.

Then add: Sodium azide 0.8 g

Allow to dissolve, adjust the volume to **200 mL** and then filter through a Whatman Polycap HD, 20 micron. Stable for > 3 years at 4°C.

NOTE:

1. If the concentrated buffer is stored below -10°C, it will form salt crystals that must be completely dissolved when this buffer is diluted to 1 L with distilled water.
2. This concentrated buffer contains 0.4% (w/v) sodium azide. This is a poisonous chemical and should be treated accordingly.

PREPARATION OF Glucose Determination Reagent:

1. Dilute 50 mL of **GOPOD Reagent Buffer** to 1 L with distilled water. **This is Solution 1**. Use immediately.
2. Dissolve the contents of bottle 2 **(GOPOD Reagent Enzymes)** 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 below -10°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°C-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°C-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:

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

	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:

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