

Megazyme

GLUCOSE ASSAY PROCEDURE

GLC 6/98

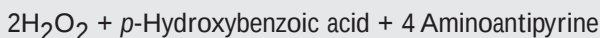
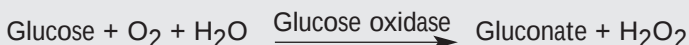


INTRODUCTION:

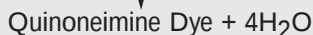
Glucose can be conveniently measured in body fluids using commercially available kits based on the glucose oxidase/peroxidase or on the hexokinase/G6PDH enzymic procedures. However, glucose in plant extracts usually occurs together with maltose, maltosaccharides, starch, sucrose and/or β -linked gluco-oligosaccharides. 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 glucose present in the extract or derived by specific enzymic degradation of glucose containing oligosaccharides or polysaccharides (e.g. barley β -glucan). Most commercially available glucose kits based on the glucose oxidase/peroxidase reaction contain reagents which are not sufficiently pure. The Megazyme Glucose Test Kit employs high purity glucose oxidase and peroxidase and can be used with confidence for the specific measurement of glucose in plant extracts. The colour which forms is stable at room temperature for at least two hours after development.

PRINCIPLE:

The reactions involved are:



↓ Peroxidase



KITS:

Kits suitable for performing 500 assays are available from Megazyme and consist of:

1. Glucose Determination Reagent (for 2 x 1 litres of reagent)
2. Glucose Reagent Buffer (for 2 x 1 litres of reagent)
3. Glucose Standard Solution

CONTENTS:

Glucose Reagent Buffer (Concentrate):

1 M Potassium dihydrogen orthophosphate

200 mM *p*-hydroxybenzoic acid

0.4% sodium azide

Store at 2-5°C (12 months) or at -20°C (>12 months).

Glucose Determination Reagent (per vial):

Glucose oxidase $\geq 12,000\text{U}$

Peroxidase $\geq 650\text{ U}$

4-Aminoantipyrine 0.4 m moles

Store at 2-5°C (12 months) or at -20°C (>12 months).

Glucose Standard:

Glucose 1.0 mg/mL

Benzoic acid 0.2% w/v

CHROMOGEN REAGENT (GOPOD) PREPARATION:

1. Dilute 50.0 mL (1 container) of the **Glucose Reagent Buffer (concentrate)** to 1 Litre with distilled water.

Note: When stored at -20°C, the buffer will crystallise. Ensure all crystalline material is dissolved in the 1 litre of distilled water.

2. Dissolve the contents of one vial of **Glucose Determination Reagent** in this buffer.
3. This **GOPOD** reagent in a brown storage bottle is stable for:
3 months at 2-5°C
>12 months in the frozen state

When this 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 (492-550)
Temperature:	40°C or 50°C
Light path:	1 cm
Zero:	Reagent Blank

ASSAY PROCEDURE:

	BLANK	STANDARD	SAMPLE
Chromogen reagent	3 mL	3 mL	3 mL
Glucose standard	-	0.1 mL	-
Sample	-	-	0.1 mL
Buffer or water	0.2 mL	0.1 mL	0.1 mL

Mix and incubate at 40 or 50°C for 20 min. Read absorbance (O.D.) at 510 nm against the **reagent blank**.

CALCULATION:

$$\text{Glucose, } \mu\text{g}/0.1 \text{ mL} = \frac{\text{O.D. Sample}}{\text{O.D. Standard (glucose, 100 } \mu\text{g)}} \times 100$$

REFERENCES:

- Trinder, P. (1969). *Ann. Clin. Biochem.*, **6**, 24.
Blakeney, A.B. and Matheson, N.K. (1984). *Starch*, **36**, 265.
McCleary, B.V. and Codd, R. (1991). *J. Sci. Food Agric.*, **55**, 303.



**Megazyme International Ireland Ltd.,
Bray Business Park, Bray,
Co. Wicklow,
IRELAND**

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

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