

Megazyme

D-GLUCOSE

ASSAY PROCEDURE (GOPOD-FORMAT)

K-GLUC 07/11

(660 Assays per Kit)



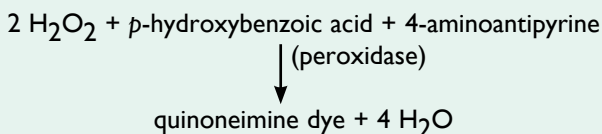
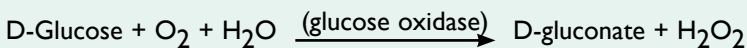
INTRODUCTION:

D-Glucose can be conveniently measured in body fluids using commercially available kits based on the glucose oxidase/oxidase 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 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/oxidase reaction contain reagents which are not sufficiently pure.

The Megazyme D-Glucose (glucose oxidase/oxidase; GOPOD) Assay Kit employs high purity glucose oxidase and oxidase 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:



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 (48 mL, pH 7.4), *p*-hydroxybenzoic acid and sodium azide (0.4 % 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 bottle 1 (GOPOD Reagent Buffer) to 1 L with distilled water. **This is Solution 1.** Use immediately.

NOTE:

1. If the concentrated buffer is stored at -20°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.

2. Dissolve the contents 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 $2-5^{\circ}\text{C}$ or > 12 months at -20°C .

If this reagent is to be stored in the frozen state, preferably it should be divided into aliquots that should be freeze/thawed only once during use.

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:	$40^{\circ}\text{C} - 50^{\circ}\text{C}$
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 $40^{\circ}\text{C} - 50^{\circ}\text{C}$ for 20 min (see table on next page). Read absorbances at 510 nm against the **reagent blank** to obtain ΔA_{sample} and $\Delta A_{\text{D-glucose standard}}$.

CALCULATION:

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

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



**Megazyme International Ireland,
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

WITHOUT GUARANTEE

The information contained in this booklet is, to the best of our knowledge, true and accurate, but since the conditions of use are beyond our control, no warranty is given or is implied in respect of any recommendation or suggestions which may be made or that any use will not infringe any patents.