

# Megazyme

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## D-GLUCOSE

### ASSAY PROCEDURE (GOPOD-FORMAT)

K-GLUC 07/11

(660 Assays per Kit)



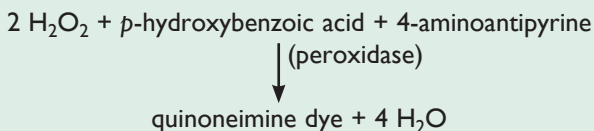
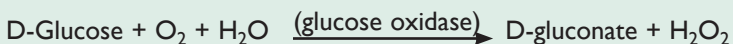
## INTRODUCTION:

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  $\beta$ -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  $\beta$ -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  $\beta$ -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  $-20^{\circ}\text{C}$ , 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  $-20^{\circ}\text{C}$ .

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  $4^{\circ}\text{C}$ . 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  $\Delta A_{\text{sample}}$  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:

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



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