

# 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)



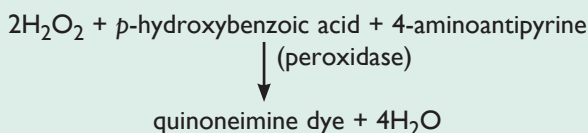
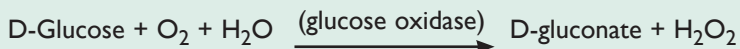
## INTRODUCTION:

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  $\beta$ -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/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:



## 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  $-10^\circ\text{C}$ .

## 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
<i>p</i> -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 I.** Use immediately.
2. Dissolve the contents of bottle 2 (**GOPOD Reagent Enzymes**) in approx. 20 mL of solution I and quantitatively transfer this to the bottle containing the remainder of solution I. 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:

<b>Wavelength:</b>	510 nm
<b>Temperature:</b>	40°C-50°C
<b>Light path:</b>	1 cm
<b>Read against:</b>	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  $\Delta A_{\text{sample}}$  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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