D-GLUCOSE

ASSAY PROCEDURE
(GOPOD-FORMAT)

K-GLUC 10/15

(660 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 overestimation or an underestimation 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 D-Glucose (glucose oxidase/peroxidase; GOPOD) Assay Kit 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:

\[
\text{D-Glucose} + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{glucose oxidase}} \text{D-gluconate} + \text{H}_2\text{O}_2
\]

\[
2 \text{H}_2\text{O}_2 + \text{p-hydroxybenzoic acid} + 4\text{-aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{quinoneimine dye} + 4 \text{H}_2\text{O}
\]

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 (50 mL, pH 7.4), p-hydroxybenzoic acid and sodium azide (0.095% 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 one of bottle 1 (GOPOD Reagent Buffer) to 1 L with distilled water. This is Solution 1. Use immediately.

   **NOTE:**
   1. On storage, salt crystals may form in the concentrated buffer. These must be completely dissolved when this buffer is diluted to 1 L with distilled water.
   2. This buffer contains 0.095% (w/v) sodium azide. This is a poisonous chemical and should be treated accordingly.

2. Dissolve the contents of one 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°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. 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:** 40-50°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 40-50°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:

\[
D-\text{Glucose (μg/0.1 mL)} = \frac{\Delta A_{\text{Sample}}}{\Delta A_{\text{D-Glucose standard (100 μg)}}} \times 100
\]

<table>
<thead>
<tr>
<th>Reagent blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>GOPOD reagent</td>
<td>3.0 mL</td>
<td>3.0 mL</td>
</tr>
<tr>
<td>D-Glucose standard sample</td>
<td>-</td>
<td>0.1 mL</td>
</tr>
<tr>
<td>buffer or water</td>
<td>0.1 mL</td>
<td>-</td>
</tr>
</tbody>
</table>

REFERENCES:


Megazyme International Ireland,
Bray Business Park, Bray,
Co. Wicklow,
A98 YV29,
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.