D-GLUCOSE
(GOPOD FORMAT)

ASSAY PROTOCOL

K-GLUC

08/23

(660 Assays per Kit)

Play Training Video

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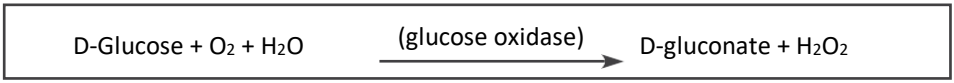
INTRODUCTION:

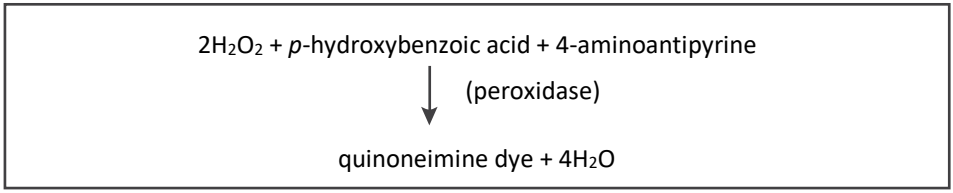
D-Glucose can be conveniently measured in body fluids using commercially available kits based on the glucose oxidase/peroxidase or the hexokinase/G6P-DH enzymic procedures. However, D-glucose in plant extracts usually occurs together with maltose, maltosaccharides, starch, sucrose and/or β-linked glucooligosaccharides. 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).

The Megazyme brand D-Glucose (glucose oxidase/peroxidase; GOPOD) Assay Kit (**K-GLUC**) 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:

Kits suitable for performing 660 assays (3 mL per assay) are available from Neogen. 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 (0.09% w/v).
Store at 4°C. See individual label for expiry date.

Bottle 2: (x 2) **GOPOD Reagent Enzymes.** Glucose oxidase plus peroxidase and 4-aminoantipyrine. Freeze-dried powder.
Store below -10 °C. See individual label for expiry date.

Bottle 3: D-Glucose standard solution (5 mL, 1.0 mg/mL) in 0.2% (w/v) benzoic acid.
Store sealed at room temperature. See individual label for expiry date.

PREPARATION OF REAGENT SOLUTIONS/SUSPENSIONS:

1. Dilute the contents of one bottle of **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 GOPOD Reagent Buffer. These must be completely dissolved when this buffer is diluted to 1 L with distilled water.
2. This buffer contains 0.09% (w/v) sodium azide. This is a poisonous chemical and should be treated accordingly.

2. Dissolve the contents of one bottle of **GOPOD Reagent Enzymes** 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 ≥ 1 month at 4°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 **GOPOD reagent** is freshly prepared it may be light yellow or light pink in colour. It may develop a stronger pink colour upon storage at 4 °C. The absorbance of this solution should be less than 0.05 when read against distilled water.

ASSAY CONDITIONS:

Wavelength:	510 nm
Cuvette:	1 cm
Temperature	40-50˚C
Final volume:	3.1 mL
Sample solution:	4-100 µg of glucose per cuvette (in 0.1 mL sample volume)
Read against:	Reagent Blank

Pipette into cuvettes	Reagent blank	Sample	Standard
GOPOD reagent	3.0 mL	3.0 mL	3.0 mL
Bottle 3 (D-Glucose standard)	-	-	0.1 mL
Sample	-	0.1 mL	-
Buffer	0.1 mL	-	-

ASSAY PROCEDURE:

NOTE: Samples containing high amounts of protein or fats may cause interference in target analyte determination and require sample clarification prior to analysis using Carrez Clarification reagents. These reagents are available to purchase separately from Neogen in the Carrez Clarification Kit (**K-CARREZ**). The **K-CARREZ** reagents should be diluted prior to use as described on page 2 of the **K-CARREZ** assay protocol and the assay procedure for clarification followed as described on page 3. The clarified sample solution prepared using the **K-CARREZ** assay kit can then be analysed for D-glucose content as described in the assay procedure for liquid samples below.

Liquid samples (e.g. Filtered Fruit Juice):

1. Clear colourless or slightly coloured solutions can be used directly in the assay (after dilution).
2. Samples should be diluted sufficiently in distilled water, to yield a D-glucose concentration of approx. 0.04-1 g/L D-glucose. (NOTE: It is recommended that the absorbance of the sample does not exceed that obtained for the D-glucose control sample. If the sample absorbance exceeds the control dilute the sample further to achieve a suitable absorbance.)
3. Add 0.1 mL of appropriately diluted liquid sample directly to the bottom of two 16 x 100 mm glass test tubes.
4. Prepare the reagent blank solutions by adding 0.1 mL of distilled water directly to the bottom of two 16 x 100 mm glass test tubes.
5. Prepare the D-glucose standards by adding 0.1 mL of **Bottle 3 (**D-glucose standard solution), directly to the bottom of 16 x 100 mm glass test tubes in quadruplicate.
6. Add 3.0 mL of **GOPOD Reagent** to all tubes and incubate these at 40-50˚C for 20 min.
7. Measure all absorbances at 510 nm against the reagent blank (*i.e.*, zero the spectrophotometer against the reagent blank)

Solid samples (e.g. Cereals or flours):

1. For solid samples grind the sample to pass a 0.5 mm screen, ensuring the sample is homogeneous.
   NOTE: The weights and volumes used in this procedure are provided as a guide only and may need to be altered depending on the sample being tested.
2. Weigh out a representative sample weight (*e.g.,* 1 g) and add to a 100 mL volumetric flask and make to the mark (*i.e.* 100 mL) with distilled water. Stir vigorously until the sample is fully dispersed or dissolved.

3. Filter and use the clear solution, diluted further (if required) for the assay. Samples should be diluted to yield a D-glucose concentration of approx. 0.04-1 g/L D-glucose. (NOTE: It is recommended that the absorbance of the sample does not exceed that obtained for the D-glucose control sample. If the sample absorbance exceeds the control dilute the sample further to achieve a suitable absorbance.)
4. Add 0.1 mL of the obtained and appropriately diluted clear solution directly to the bottom of two 16 x 100 mm glass test tubes.
6. Prepare the reagent blank solutions by adding 0.1 mL of distilled water directly to the bottom of two 16 x 100 mm glass test tubes.
7. Prepare the D-glucose standards by adding 0.1 mL of **Bottle 3** (D-glucose standard solution), directly to the bottom of 16 x 100 mm glass test tubes in quadruplicate.
8. Add 3.0 mL of **GOPOD Reagent** to all tubes and incubate these at 40-50˚C for 20 min.
9. Measure all absorbances at 510 nm against the reagent blank (*i.e.*, zero the spectrophotometer against the reagent blank).

CALCULATION:

NOTE: These calculations can be simplified by using the Megazyme ***Mega-Calc***™, downloadable from where the product appears on the Megazyme website (www.megazyme.com).

The concentration of glucose can be calculated as follows:

$$\mathbf{c}\ (\mathbf{g/L}) = \Delta A_{Sample} \times \frac{100}{\Delta A_{Standard}} \times 10 \times \frac{1}{1000} \times \frac{1000}{1000} \times \text{Dilution}$$

Therefore;

$$\mathbf{c}\ (\mathbf{g/L}) = \Delta A_{Sample} \times \frac{1}{\Delta A_{Standard}} \times \text{Dilution}$$

where:

ΔA_{Sample} = absorbance of sample read against the reagent blank
100 = 100 µg of D-glucose used as standard
$\Delta A_{Standard}$ = absorbance of 100 µg of D-glucose standard read against the reagent blank
10 = conversion from 0.1 mL of sample assayed to 1 mL
1/1000 = conversion from µg to mg
1000/1000 = conversation from mg/mL to g/L

Dilution = further dilution prior to incubation with GOPOD Reagent (if required).

When analysing solid and semi-solid samples which are weighed out for sample preparation, the content (g/100 g) is calculated from the amount weighed as follows:

Content of glucose

$$c = \frac{C_{glucose}\ [g/L\ sample\ solution]}{weight_{sample}\ [g/L\ sample\ solution]} \times 100 \quad [g/100\ g]$$

REFERENCES:

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3. McCleary, B. V. & Codd, R. Measurement of (1→3),(1→4)-β-D-glucan in barley and oats: A streamlined enzymic procedure. (1991). *J. Sci. Food Agric.*, **55**, 303.

Contact us for more information: neogen.com/contact

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