

GLUCOSE OXIDASE

ASSAY PROTOCOL

K-GLOX

08/23

(200 Manual Assays per Kit) or
(1960 Auto-Analyser Assays per Kit) or
(2000 Microplate Assays per Kit)



INTRODUCTION:

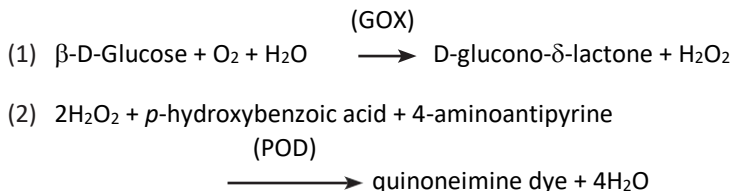
Glucose oxidase (GOX) catalyses the oxidation of β -D-glucose to D-glucono- δ -lactone and hydrogen peroxide. It is highly specific for β -D-glucose and does not act on α -D-glucose.

A major use of glucose oxidase has been in the determination of free glucose in body fluids, food and agricultural products. It is often also used in the baking industry where addition results in a stronger dough. In some applications it can be used to replace oxidants, such as bromate and L-ascorbic acid. Other uses of glucose oxidase include the removal of oxygen from food packaging and removal of D-glucose from egg white to prevent browning.

PRINCIPLE:

Glucose oxidase catalyses the oxidation of β -D-glucose to D-glucono- δ -lactone with the concurrent release of hydrogen peroxide (1). In the presence of peroxidase (POD), this hydrogen peroxide (H_2O_2) enters into a second reaction involving *p*-hydroxybenzoic acid and 4-aminoantipyrine with the quantitative formation of a quinoneimine dye complex which is measured at 510 nm (2).

The reactions involved are:



SAFETY:

The general safety measures that apply to all chemical substances should be adhered to. For more information regarding the safe usage and handling of this product please refer to the associated SDS that is available from the Megazyme website.

KITS:

Kits suitable for performing 200 assays in manual format (or 1960 assays in auto-analyser format or 2000 assays in microplate format) are available from Neogen. The kits contain the full assay method plus:

Bottle 1: (x 2) Buffer (12 mL, pH 7.0) plus *p*-hydroxybenzoic acid and sodium azide (0.09% w/v) as a preservative.
Store at 4°C. See individual label for expiry date.

- Bottle 2: (x 2)** Peroxidase plus 4-aminoantipyrine and stabilisers; lyophilised powder. Store below -10°C. See individual label for expiry date.
- Bottle 3:** D-Glucose (~ 10 g).
Store sealed at room temperature. See individual label for expiry date.
- Bottle 4:** Glucose oxidase standard (approx. 2.9 U; actual value stated on the vial label).
Store below -10°C. See individual label for expiry date.
- Bottle 5:** Buffer (5 mL, pH 7.0) plus BSA (0.5% w/v) and sodium azide (0.04% w/v) as a preservative.
Store at 4°C. See individual label for expiry date.

PREPARATION OF REAGENT SOLUTIONS:

1. Dilute the contents of one of **bottle 1** to approx. 150 mL with distilled water. This is **solution 1**. Use immediately. Do not dilute the contents of the second bottle until required.
2. Dissolve the contents of one of **bottle 2** in **solution 1**. Check the pH and, if necessary, adjust to pH 7.0 with 1 M HCl or 1 M NaOH. Adjust the volume to 200 mL. This is **solution 2** (POD mixture).
Stable for ≥ 1 month at 4°C or ≥ 12 months below -10°C.

If this reagent is to be stored frozen it should be divided into suitably sized aliquots and stored in polypropylene containers. Do not apply these stored solutions to more than one freeze-thaw cycle.
3. Weigh 4.5 g of the contents of **bottle 3** (D-glucose) and dissolve in 40 mL of distilled water. Adjust the volume to 50 mL. This is **solution 3** (D-Glucose).
Stable for ≥ 12 months below -10°C.
- 4.& 5. Dilute the contents of **bottle 5** to approx. 40 mL with distilled water. This is **solution 4**. Use ~ 3 mL of **solution 4** to dissolve the contents of **bottle 4**. Quantitatively transfer the dissolved contents of **bottle 4** to a 50 mL volumetric flask and use ~ 5 mL of **solution 4** to rinse the contents of **bottle 4** into the volumetric flask. Add the remainder of **solution 4** to the volumetric flask and adjust the volume to 50 mL with distilled water. Divide into 10 mL aliquots and store in polypropylene containers. This is **solution 5** (Glucose oxidase standard).
Stable for ≥ 2 years below -10°C.

NOTE: The glucose oxidase standard (**solution 5**) is only assayed in the manual procedure (A.) where there is some doubt about the accuracy of the spectrophotometer being used or where it is suspected that inhibition is being caused by substances in the sample. When performing procedures B. or C. the use of **solution 5** is required.

EXTRACTION/DILUTION BUFFER (Buffer A):

0.1 M Potassium phosphate buffer (pH 7.0) containing 0.5 mg/mL BSA and 0.02% (w/v) sodium azide as a preservative.

Add 13.6 g of potassium dihydrogen orthophosphate (MW = 136.1) to 800 mL of distilled water and adjust to pH 7.0 with 2 M sodium hydroxide. Adjust the volume to 1 L. Add 0.5 g of BSA (Sigma cat. no. A2153) and 0.2 g of sodium azide (Sigma cat. no. S2002) and dissolve. This is **buffer A**.

EQUIPMENT (RECOMMENDED):

1. Volumetric flasks (50 mL and 100 mL).
2. Disposable plastic cuvettes (1 cm light path, 3.0 mL).
3. Micro-pipettor, e.g. Gilson Pipetman® (1000 µL).
4. Positive displacement pipettor, e.g. Eppendorf Multipette®
- with 25 mL Combitip® [to dispense 0.5 mL aliquots of **solution 3** (D-glucose) and 2.0 mL aliquots of **solution 2** (POD mixture)].
5. Analytical balance.
6. Recording spectrophotometer set at 510 nm and at $25 \pm 0.1^\circ\text{C}$.
7. Vortex mixer (e.g. IKA® Yellowline Test Tube Shaker TTS2).

SAMPLE PREPARATION EXAMPLE:

Determination of glucose oxidase in enzyme preparations. Accurately weigh approx. 100 mg of glucose oxidase preparation into a 100 mL beaker. Add 50.0 mL of extraction/dilution buffer (**buffer A**) and gently stir the preparation on a magnetic stirrer until the enzyme completely dissolves. Dilute this solution by transferring 1.00 mL into a 100 mL volumetric flask and adjusting to volume with **buffer A**. Mix well by inversion and dilute further as necessary to obtain an enzyme concentration suitable for assay (0.01-0.08 U/mL).

A. MANUAL ASSAY PROCEDURE:

Wavelength: 510 nm
Cuvette: 1 cm light path (glass or plastic)
Temperature: $25 \pm 0.1^\circ\text{C}$
Final volume: 3.0 mL
Sample solution: glucose oxidase diluted to 0.01-0.08 U/mL
Read against air (without a cuvette in the light path) or against water

Pipette into cuvettes	Blank	Sample
solution 2 (POD mixture)	2.00 mL	2.00 mL
solution 3 (D-glucose)	0.50 mL	0.50 mL
Mix*, read the absorbances of the solutions (A_1) after approx. 5 min and start the reactions by addition of:		
sample solution	-	0.50 mL
distilled water	0.50 mL	-
Mix* and read the absorbances of the solutions (A_2) after exactly 20 min.		

* for example with a plastic spatula or by gentle inversion after sealing the cuvette with a cuvette cap or Parafilm®.

CALCULATION:

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

Determine the absorbance difference ($A_2 - A_1$) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{510 \text{ nm}}/20 \text{ min}$ for the sample being analysed. While the time course increase in absorbance on incubation of glucose oxidase with the assay reagents is linear (**Figure 1**, page 5), the standard curve relating glucose oxidase activity (mU/assay i.e./0.5 mL) to absorbance increase in 20 min at 510 nm is not perfectly linear (**Figure 2**, page 6). Consequently, activity values (mU/0.5 mL) are obtained by reference to the standard curve shown in **Figure 2**, and calculated as follows:

$$\begin{aligned} \text{U/L of sample solution} &= \text{mU}/0.5 \text{ mL} \times 2000 \times \frac{1}{1000} \times D \\ &= \text{mU}/0.5 \text{ mL} \times 2 \times D \end{aligned}$$

where:

mU/0.5 mL is obtained from the $\Delta A_{510 \text{ nm}}/20 \text{ min}$, by reference to the standard curve (**Figure 2**, page 6)

2000 = conversion from 0.5 mL as assayed to 1 L

1/1000 = conversion from mU to U

D = dilution factor (i.e. if sample is diluted 10-fold, D=10)

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

Glucose oxidase activity (U/g of preparation)

$$= \frac{\text{GOX activity [U/L sample solution]}}{\text{weight}_{\text{sample}} [\text{g/L sample solution}]} \quad [\text{U/g}]$$

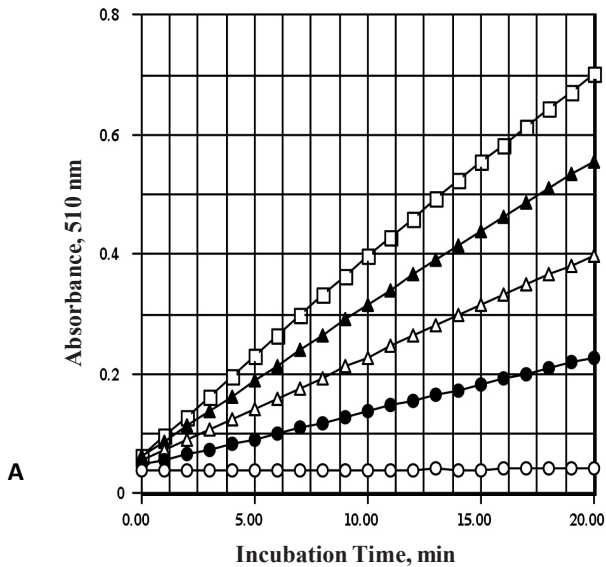


Figure 1. Linearity of the reaction curves for various concentrations of glucose oxidase in the assay mixture. **A.** 0 mU; **B.** 10 mU; **C.** 20 mU; **D.** 30 mU; **E.** 40 mU.

$$\text{mUnits}/0.5 \text{ mL} = (15.4 \times \text{Abs}^2) + (44.7 \times \text{Abs}) + 0.03$$

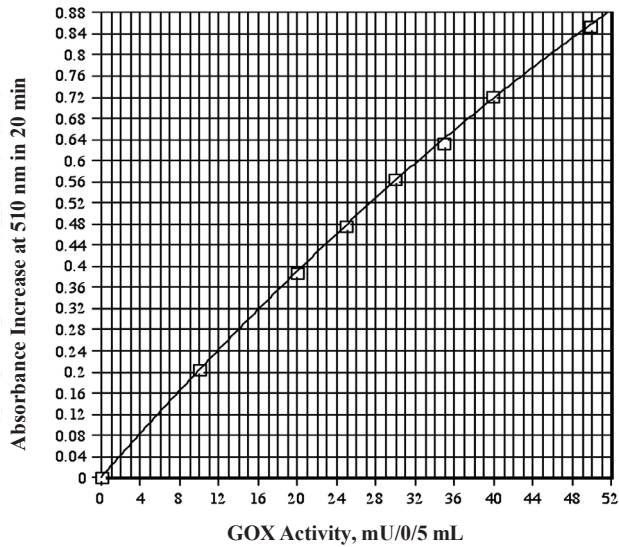


Figure 2. Standard curve relating glucose oxidase activity (mU/assay i.e./0.5 mL) to absorbance at 510 nm.

B. AUTO-ANALYSER ASSAY PROCEDURE:

NOTES:

1. The Auto-Analyser Assay Procedure for glucose oxidase can be performed using either a single point standard or a full calibration curve.
2. For each batch of samples that is applied to the determination of glucose oxidase **either a single point standard or a calibration curve must be performed concurrently using the same batch of reagents.**

Reagent preparation is performed as follows:

Preparation of R1:

Component	Volume
solution 2 (POD mixture)	100 mL
solution 3 (D-glucose)	25 mL
Total volume	125 mL

EXAMPLE METHOD:

R1: 0.250 mL

Sample: 0.050 mL

Reaction time: 20 min at 25°C

Wavelength: 510 nm

Prepared reagent stability: > 2 days when refrigerated

Calculation: kinetic

Reaction direction: increase

Linearity: 0.01-0.08 U/mL of glucose oxidase using 0.01 mL sample volume

C. MICROPLATE ASSAY PROCEDURE:

NOTES:

1. The Microplate Assay Procedure for glucose oxidase can be performed using either a single point standard or a full calibration curve.
2. For each batch of samples that is applied to the determination of glucose oxidase **either a single point standard or a calibration curve must be performed concurrently using the same batch of reagents.**

Wavelength: 510 nm
Microplate: 96-well (e.g. clear flat-bottomed, glass or plastic)
Temperature: 25°C
Final volume: 0.300 mL
Linearity: 0.01-0.08 U/mL of glucose oxidase per well
(in 0.05 mL sample volume)

Pipette into wells	Blank	Sample	Standard
solution 2 (POD mixture) solution 3 (D-glucose)	0.200 mL 0.050 mL	0.200 mL 0.050 mL	0.200 mL 0.050 mL
Mix*, read the absorbances of the solutions (A_1) after approx. 5 min and start the reactions by addition of:			
sample solution solution 5 (Glucose oxidase standard) distilled water	- - 0.050 mL	0.050 mL - -	- 0.050 mL -
Mix* and read the absorbances of the solutions (A_2) after exactly 20 min.			

* for example using microplate shaker, shake function on a microplate reader or repeated aspiration (e.g. using a pipettor set at 50-100 μ L volume).

CALCULATION (Microplate Assay Procedure):

Determine the absorbance difference ($A_2 - A_1$) for blank, samples and calibration curve standards (or single point standard). Subtract the absorbance difference of the blank from the samples and standards to obtain $\Delta A_{510 \text{ nm}}/20 \text{ min}$. Test samples values are obtained by direct cross reference to the calibration curve of glucose oxidase activity vs $\Delta A_{510 \text{ nm}}/20 \text{ min}$.

Alternatively:

$$\text{U/L} = \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times \text{U/L standard} \times D$$

If the sample is diluted during preparation, the result must be multiplied by the dilution factor, D.



Contact us for more information: neogen.com/contact

Without guarantee

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