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Product Information

o-Dianisidine Dihydrochloride
suitable for use in glucose determination

Catalog Number **F5803**
Storage Temperature 2–8 °C

Product Description

This product is a preweighed vial containing 50 mg of *o*-dianisidine dihydrochloride, which is a peroxidase substrate used in the quantitative determination of glucose in aqueous solutions such as serum, using a coupled enzyme system with glucose oxidase and peroxidase (PGO enzymes).

The procedure is based upon the following coupled enzymatic reactions:[1-5]

$$\text{Glucose} + 2H_2O + O_2 \xrightarrow[\text{Oxidase}]{\text{Glucose}} \text{Gluconic Acid} + 2H_2O_2$$

$$H_2O_2 + \underset{\text{(Colorless)}}{\textit{o}\text{-Dianisidine}} \xrightarrow{\text{Peroxidase}} \underset{\text{(Brown)}}{\text{Oxidized } \textit{o}\text{-Dianisidine}}$$

The reactions are normally monitored at 425–475 nm when utilizing *o*-dianisidine as a colorimetric substrate. The intensity of the brown color measured at 425–475 nm is proportional to the original glucose concentration.

o-Dianisidine (3,3′-dimethoxybenzidine) is also used as a peroxidase substrate in ELISA procedures. This substrate produces a soluble end product that is yellow-orange in color and can be read spectrophotometrically at 405 nm. The reaction may be stopped with 5 M HCl.

Reagents Required but Not Provided

- PGO Enzymes (Catalog Number P7119)
- Glucose Standard Solution, 1 mg/ml (Catalog Number G6918)
- Optional reagent: Barium hydroxide solution, 0.3 N (Catalog Number B4059)
- Optional reagent: Zinc sulfate solution, 0.3 N (Catalog Number Z2876)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Prepare the *o*-Dianisidine Solution by reconstituting one vial of *o*-dianisidine dihydrochloride (Catalog Number F5803) with 20 ml water.

Prepare the PGO Enzymes Solution by adding the contents of 1 capsule of PGO Enzymes (Catalog Number P7119) to 100 ml of water in an amber bottle. Invert bottle several times with gentle shaking to dissolve.

Prepare the PGO Enzymes Reaction Solution by combining 100 ml of the PGO Enzyme Solution with 1.6 ml of the *o*-Dianisidine Solution. Mix by inverting several times or with mild shaking.

Dilute 5.00 ml of Glucose Standard Solution to 100.0 ml with water (20-fold dilution) to prepare a glucose solution of 0.05 mg/ml.

Storage/Stability

Store *o*-Dianisidine hydrochloride at 2–8 °C.

Reconstituted *o*-Dianisidine hydrochloride solution is stable for 3 months when stored at 2–8 °C.

Procedures

The glucose-containing sample is added to the PGO Enzymes Reaction Solution. The reaction proceeds to completion in ~30 minutes at 37 °C. The final absorbance is proportional to the glucose concentration. A distinct advantage of this procedure is precise timing of the reaction is not necessary.

1. Label three or more tubes or cuvettes as follows: Blank, Standard, Test 1, Test 2, etc. (as needed).
2. Add 0.5 ml of water to the Blank tube. Add 0.5 ml of the prepared 0.05 mg/ml glucose standard solution to the Standard tube. Add 0.5 ml of sample to each tube marked Test. Typically, serum samples should be diluted 20-fold.
3. To each tube add 5.0 ml of the PGO Enzymes Reaction Solution and mix each tube thoroughly.
4. Incubate all tubes at 37 °C for 30±5 minutes or at room temperature (18–26 °C) for 45 minutes.
   Note: Avoid exposure to direct sunlight or bright daylight during incubation.
5. At the end of incubation period, remove all tubes from the water bath. Read the absorbance (A) at 425–475 nm of the Standard and Tests, using the Blank as the reference.
   Note: Readings should be completed within 30 minutes.

Alternative Procedure

This procedure is used when determining glucose content of whole blood, or colored or turbid serum or plasma. A deprotinization step is included to reduce sample interference.

1. Label three or more tubes or cuvettes as follows: Blank, Standard, Test 1, Test 2, etc. (as needed).
2. Add 1.8 ml of water to each tube.
3. Add 0.2 ml of water to the Blank tube. Add 0.2 ml of the 1 mg/ml Glucose Standard Solution (Catalog Number G6918) to the Standard tube.
4. Add 0.2 ml of blood, plasma, or serum to each tube marked Test. Swirl to mix and hemolyse the blood.
   a. Add 1.0 ml of 0.3 N Barium Hydroxide Solution (Catalog Number B4059) to each tube and swirl to mix.
   b. Ad 1.0 ml of 0.3 N Zinc Sulfate Solution (Catalog Number Z2876) to each tube. Stopper tightly and mix well. Barium sulfate will precipitate.
   c. Centrifuge or filter each tube to obtain a clear supernatant/filtrate.
5. Transfer 0.5 ml of clear supernatant/filtrate from each tube to another set of clean dry tubes or cuvettes. Label second set of tubes to correspond with first set (Test 1, Test 2, etc.).
6. Transfer 0.5 ml of Standard and Blank solutions to clean dry tubes or cuvettes. Label correspondingly as Standard and Blank.
7. To each of the second set of tubes, add 5.0 ml of the PGO Enzymes Reaction Solution. Mix each tube thoroughly.
8. Incubate all tubes at 37 °C for 30±5 minutes or at room temperature (18–26 °C) for 45 minutes.
   Note: Avoid exposure to direct sunlight or bright daylight during incubation.
9. At the end of incubation period, remove all tubes from water bath. Read the absorbance (A) at 425–475 nm of the Standard and Tests, using the Blank as the reference.
   Note: Readings should be completed within 30 minutes.

Results

Calculations

The glucose concentration of the sample is determined as follows:

Sample Glucose Concentration (mg/ml) =

$$\frac{\text{Absorbance (A) Test} \times \text{Dilution of sample} \times 0.05 \text{ mg/ml}}{\text{Absorbance (A) Standard}}$$

For the alternative procedure with deproteinization by precipitation:

Sample Glucose Concentration (mg/ml) =

$$\frac{\text{Absorbance (A) Test} \times 1 \text{ mg/ml}}{\text{Absorbance (A) Standard}}$$

References

1. Henry, J.B., Clinical Chemistry, in Todd-Sanford, Clinical Diagnosis by Laboratory Methods, 15th ed. Davidsohn, I., and Henry, J.B., eds, Saunders, (Philadelphia, PA: 1974) pp 601–612.
2. Keston, A.S., Specific colorimetric enzymatic analytical reagents for glucose. Abstract of Papers, 129th Meeting, ACS, Dallas (TX), April 1956, p 31C.
3. Keilin, D., and Hartree, E.F., Properties of glucose oxidase (notatin). Biochem. J., **42**, 221 (1948).
4. Keilin, D., and Hartree, E.F., Specificity of glucose oxidase (notatin). Biochem. J., **50**, 331 (1952).
5. McComb, R.B., et al., 2-Deoxy-D-glucose, a new substrate for glucose oxidase (glucose aerodehydrogenase). J. Franklin Inst., **263**, 161 (1957).

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