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Assay method of glucose oxidase activity

葡萄糖氧化酶活性检测方法

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Foreword

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This document was proposed by and shall be under the jurisdiction of National Technical Committee 11 on Research Use Enzyme of Standardization Administration of China (SAC/SWG 11).

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Assay Method of Glucose Oxidase Activity

1 Scope

This document describes the assay method of glucose oxidase activity.

This document applies to the assay of glucose oxidase activity.

2 Normative References

GB/T 6682

3 Terms and Definitions

The following terms and definitions are applicable to this document.

3.1 Glucose oxidase

Under the condition where there is oxygen, a dehydrogenase that specifically catalyzes beta-D- glucose to generate D-gluconic acid and hydrogen peroxide.

3.2 Activity unit of glucose oxidase

At pH 5.1 and 35 °C, the amount of enzyme required to oxidize 1.0 ?mol of beta-D-glucose to D- gluconic acid and hydrogen peroxide per minute is defined as one activity unit (U).

4 Principle

Under the action of glucose oxidase, glucose reacts with oxygen to generate gluconic acid and hydrogen peroxide. Hydrogen peroxide reacts with colorless reduced o-dianisidine under the action of peroxidase to generate water and red oxidized o-dianisidine. This red substance has a specific absorption peak at a wavelength of 500 nm. By determining the rate of increase in absorbance at 500 nm of the red substance, the number of activity units of the enzyme can be calculated.

5.1 Water

Grade-2 water conforming to the provisions of GB/T 6682.

5.2 50 mmol/L Sodium Acetate Buffer Solution Weigh-take 0.82 g of anhydrous sodium acetate and use 150 mL of pure water to dissolve it. At 35 °C, use 1 mol/L hydrochloric acid to adjust the pH to 5.1 and reach a constant volume of 200 mL. 5.3 0.21 mmol/L o-Dianisidine Weigh-take 50 mg of o-dianisidine dihydrochloride and use 7.5 mL of pure water to dissolve it.

Take 1 mL and use 50 mmol/L sodium acetate buffer solution (pH 5.1) to dilute it and reach a constant volume of 100 mL.

5.4 10% beta-D(+) Glucose Solution Weigh-take 1 g of beta-D(+) glucose, use pure water to dissolve it and reach a constant volume of 10 mL.

5.5 0.17 mmol/L o-Dianisidine-1.72% Glucose Solution Take 24 mL of 0.21 mmol/L o-Dianisidine and 5 mL of 10% ?-D(+) glucose solution, mix well, and keep it at a constant temperature of 35 ?C for later use. Prepare it right before use.

5.6 Horseradish Peroxidase Solution

Take an appropriate amount of horseradish peroxidase, use ice pre-cooled pure water to prepare it and dilute it to a solution of 60 red phenol units per mL (U/mL).

5.7 Test Sample Solution

Weigh-take an appropriate amount of the test sample solid or solution, use ice pre-cooled 50 mmol/L sodium acetate buffer solution (pH 5.1) to dissolve it and dilute to a solution of 0.4 U/mL ~ 0.8 U/mL.

6.1 Substrate Solution

In accordance with Table 1, prepare the substrate working solution, and maintain it in a water bath at 35 ?C for 5 min. .....