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LACTATE OXIDASE

Principle：

The assay is based on the increase in absorbance at 565 nm as the formation of quinoneimine dye proceeds in the following reactions：

$$\text{L-Lactate} + O_2 \xrightarrow{\text{LOX II}} \text{Pyruvate} + H_2O_2$$

$$2\,H_2O_2 + \text{4-AA} + \text{DMA} + H^+ \xrightarrow{\text{POD}} \text{Quinonediimine dye}^+ + 4\,H_2O$$

DMA: N, N'-Dimethylaniline

Unit definition：

One unit is defined as the amount of enzyme which generates 1 μ mole of H2O2 per minute at 37℃ under the conditions specified in the assay procedure.

Reagents：

1. Reaction mixture

0.2 M KH2PO4–NaOH buffer pH 6.5——0.20 ml；

50 U/ml POD solution [1)] ——0.10 ml；

15 mM 4–AA solution——0.10 ml；

0.5 M DL–Lactic acid solution pH 6.5——0.10 ml；

Distilled water——0.30 ml；

Mix above reagents in advance. Just before measuring, add the reagent listed below and mix.

0.2%（W/V）DMA solution——0.20 ml；

1）: 50 U/ml POD solution——Dissolve 500 U（PPU）of POD with 10 ml of distilled water.

2. Reaction stopper

0.25%（W/V）LBS solution

LBS: Sodium lauryl sulfate

3. Enzyme dilution buffer

10 mM KH2PO4 –NaOH buffer pH 7.0 containing

10 μM FAD

FAD: Flavine adenine dinucleotide

4. Reagents

DL–Lactate.；DMA；FAD（2Na）；LBS；4–AA；POD。

Enzyme solution：

Accurately weigh about 20 mg of the sample and add enzyme dilution buffer to make a total of 20 ml. Dilute it with enzyme solution buffer to adjust the concentration as required.

Procedure：

1. Pipette accurately 1.0 ml of reaction mixture into a small test tube and preincubate at 37℃.

2. After 5 min, add exactly 20 μl of enzyme solution and mix to start the reaction at 37℃.

※ In the case of a test blank, add 20 μl of enzyme dilution buffer in place of enzyme solution.

3. At 10 min after starting the reaction, add 2.0 ml of the reaction stopper to stop the reaction.

4. Measure the absorbance at 565 nm.

Absorbance：

sample : As

blank : Ab

△A =（As−Ab）≦ 0.350 Abs

Calculation：

$$\text{Activity（U/mg of powder）} = \frac{\Delta A/10}{35.33 \times 1/2} \times \frac{3.02}{0.02} \times \frac{1}{X}$$

35.33 : millimolar extinction coefficient of quinonediimine dye at 565 nm（cm2 / μmole）

1/2 : a multiplier derived from the fact that 2 mole of H_2O_2 produces 1 mole of quinonediimine dye

10 : reaction time（min）

3.02 : final volume（ml）

0.02 : volume of enzyme solution（ml）

X : concentration of the sample in enzyme solution （mg/ml）

Storage：

Storage at −20℃ in the presence of a desiccant is recommended. The enzyme activity will be retained for at least one year under this condition.