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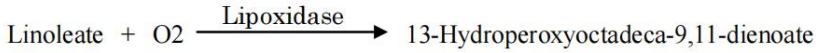
脂肪氧化酶

1. Titer Measurement

(1) Preparation

Reaction scheme：



Unit Definition：

That amount of enzyme causing an increase in extinction at 234nm of 0.001per minute at pH 9.0 and 25deg-C

Reagent preparation：

a. Boric acid buffer (H3BO3-KCl-NaOH pH 9.0)

Dissolve 1.14g of Boric acid and 1.49g of KCl in deionized water and dilute to 100mL.

Adjust pH to 9.0 by 0.2M NaOH aq.

b. Substrate solution

Dissolve 50.48mg of Linoleic Acid (use high purity grade) in adequate amount of the Boric acid buffer prepared at step a., add 1mL of Ethanol and dilute to exactly 10mL with the Boric acid buffer----------stock solution x10 substrate solution : stock solution 1mL + EtOH 0.9mL + Boric acid buffer 8.1mL

c. Enzyme solution

Dissolve 10mg of Lipoxidase in 10mL of the Boric acid buffer. Dilute this solution to x10, x20 step by step and then prepare x200 enzyme solution (dilute solution; Boric acid buffer).

(2) Operation

1. By bubbling O2 gas for few min using a balloon, let the x10 substrate solution absorbed O2*.

2. Set a spectrophotometer to 234nm

3. Put 2.0mL of the previously O2 bubbled substrate solution in a quartz cell

4. Measure the temperature surrounding the spectrophotometer and adjust the temperature to 25deg-C

5. When all condition mentioned above is ready, add 1mL of x 200 enzyme solution to the substrate solution, mix thoroughly and record abs increase per minute for 10 minutes. Measure the change in absorbance per min over liner portion of the curve and this value in the calculation.

*perform right before the measurement

(3) Calculation* 2

$$U/mg = \frac{\Delta E234nm/\min}{0.001} \times \frac{1}{a}$$

Where,

U : Enzyme unit(U/mg)

ΔE234nm/min : Change in abs per min

a : Amount of sample used in the test

0.001 : See unit definition