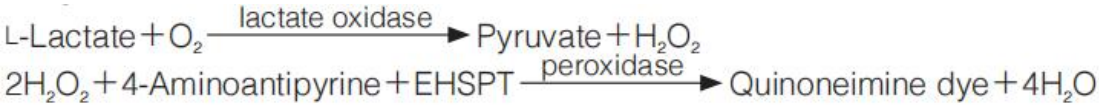
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乳酸氧化酶

Principle：



The appearance of quinoneimine dye is measured at 555nm by spectrophotometry.

Unit definition：

One unit causes the formation of one micromole of hydrogen peroxide (half a micromole of quinoneimine dye) per minute under the conditions described below.

Method：

Reagents

A. DL-Lactate solution ： 0.125M [240mg of DL-lithium lactate (MW=96.01)/20ml of 50mM K-PB pH7.5] (Should be prepared fresh)

B. 4-AA solution：0.5% (500mg of 4-aminoantipyrine/100ml of H2O) (Store at 4℃ in a brownish bottle)

C. EHSPT(TOOS) solution ： 20mM [591mg N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine (MW=295.3)/100ml of H2O] (Store at 4℃ in a brownish bottle)

D. Peroxidase solution： 25U/ml [ca. 23mg of horseradish peroxidase (Toyobo GradeⅢ, 110 purpurogallin units/mg)/100 ml of H2O]

E. SDS solution：0.25% (500mg sodium dodecyl sulfate/200ml of H2O)

F. Enzyme diluent：20mM K-PB, pH7.0 containing 0.1%(w/v) sodium cholate

Procedure：

1. Prepare the following working solution (20 tests) in a brownish bottle, and store on ice.

(A)8.0ml，DL-Lactate solution；

(B)1.2ml：4-AA solution；

(C)0.8ml：EHSPT solution；

(D)2.0ml：Peroxidase solution；

8.0ml：distilled water。

Concentration in assay mixture	
K-phosphate buffer	20 mM
DL-Lactate	48 mM
4-Aminoantipyrine	1.2 mM
EHSPT	0.76 mM
Peroxidase	2.4 U/ml

2. Pipette 1.0ml of working solution into a test tube and equilibrate at 37℃ for about 5 minutes.

3. Add 0.05ml of the enzyme solution* and mix.

4. After exactly 15minutes at 37℃, add 2.0ml of SDS solution (E) to stop the reaction and measure the optical density at 555nm against water (ODtest).

At the same time, prepare the blank by using the same method as the test except that the enzyme diluent (F) is used instead of the enzyme solution (ODblank).

* Dissolve the enzyme preparation in ice-cold 20mM ACES-NaOH pH7.0 containing 1mM EDTA and 0.5%(w/v) sodium cholate, and dilute to 0.04−0.1 U/ml with the enzyme diluent (F) immediately before assay.

Calculation：

Activity can be calculated by using the following formula：

$$\text{Volume activity (U/ml)} = \frac{\Delta OD\ (OD\ test - OD\ blank) \times Vt \times df}{34.3 \times 1/2 \times t \times 1.0 \times Vs} = \Delta\ OD \times 0.237 \times df$$

Weight activity (U/mg) ＝ (U/ml)×1/C

Vt : Total volume (3.05ml)

Vs : Sample volume (0.05ml)

34.3 : Millimolar extinction coefficient of quinoneimine dye under the assay condition (cm^2/micromole)

1/2 : Factor based on the fact that one mole of H_2O_2 produced half a mole of quinoneimine dye

t : Reaction time (15minutes)

1.0 : Light path length (cm)

C : Enzyme concentration in dissolution (C mg/ml)