



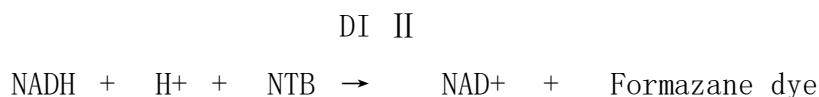
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DIAPHORASE (NADH) [DI II]

ASSAY

Principle

The assay is based on the increase in absorbance at 550 nm as the formation of formazane dye (NTBH₂) proceeds in the following reaction:



NADH: Nicotineamido adenine dinucleotide

NTB: Nitrotetrazolium blue

REAGENTS

1、Reaction mixture

| | |
|---|---------|
| 0.2 M KH ₂ PO ₄ – NaOH buffer | 0.50 ml |
| pH 8.0 | |
| 0.25% (W/V) NTB solution | 0.10 ml |
| 1% (W/V) BSA solution | 0.10 ml |
| 10 mM NADH solution | 0.10 ml |
| Distilled water | 0.20 ml |

2、Reaction stopper: 0.1 N HCl solution

3、Enzyme dilution buffer

0.1 M KH₂PO₄–NaOH buffer pH8.0 containing 0.1%(w/v) BSA



4、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 dilution 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°C.

2、After 5 min, add exactly 100 μ l of enzyme solution and mix to start the reaction at 37°C.

※ In the case of a test blank, add 100 μ l 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 550 nm.

Absorbance sample : A_s

blank : A_b

$$\Delta A = (A_s - A_b) \leq 0.370 \text{ Abs}$$

CALCULATION



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Activity (U/mg of powder) = $\{(\Delta A/10)/12.4\} \times 3.10/0.10 \times 1/x$

12.4 : millimolar extinction coefficient of Formazane dye at 550 nm

(cm²/ μmole)

10 : reaction time (min)

3.10 : final volume (ml)

0.10 : volume of enzyme solution (ml)

X : concentration of the sample in enzyme solution

(mg/ml)