上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：021-61312973　　传真：021-55068248
网址：www.shyuanye.com
邮箱：shyysw@sina.com

DIAPHORASE（NADH）[DIⅡ]

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:

$$NADH + H^+ + NTB \xrightarrow{DI\ II} NAD^+ + \text{Formazane dye}$$

NADH: Nicotineamido adenine dinucleotide

NTB: Nitrotetrazolium blue

REAGENTS

1、Reaction mixture

0.2 M KH_2PO_4－NaOH buffer pH 8.0	0.50 ml
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 KH_2PO_4–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℃.

2、After 5 min, add exactly 100 μl of enzyme solution and mix to start the reaction at 37℃.

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

Absorbance sample : As
blank : Ab

△ A = (As−Ab) ≦ 0.370 Abs

CALCULATION

Activity（U/mg of powder）= {(△A/10)/12.4} × 3.10/0.10 × 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）