Telephone
+27-21-691-2020

FAIZYME LABORATORIES

Fax
+27-21-691-2025

PO Box 24516 Lansdowne 7779 Cape Town South Africa

ASSAY / ANALYTICAL PROCEDURE
DEOXYRIBONUCLEASE_KUNITZ

1. METHOD OF ASSAY:

Based on that of KUNITZ in which the rate of increase in absorbance caused by the Hydrolysis of deoxyribonucleic acid (DNA) is measured spectrophotometrically at 260nm and 25^{O}C.

2. UNIT DEFINITION:

That amount of enzyme which causes an increase of absorbance at 260nm of 0,001 per minute at 25^{O}C.

3. REAGENTS:

3.1 **1,0 M Acetate Buffer pH 5,0**

3.1.1 Dissolve 13,6 g CH_3 COONa . $3H_2O$ [MM 136,1] in distilled H_2O and adjust volume to 100ml.

3.1.2 Dissolve 5,7 ml glacial acetic acid [MM 60,01] in distilled H_2O and dilute to 100ml.

3.1.3 Titrate solution 1 with solution 2 to yield buffer at pH5,0.

3.2 **0,05 M Magnesium Sulphate Solution**

Dissolve 1,23g $MgSO_4$.7 H_2O [MM 246,48] in distilled H_2O and adjust volume to 100ml.

3.3 **Substrate 4mg % DNA/0,005 M $MgSO_4$/0,1 M Acetate Buffer pH5,0**

Dissolve 4mg DNA in 50ml distilled H_2O. Store overnight at 5^{O}C. Add 10ml buffer [3.1] and 10ml $MgSO_4$ [3.2] and adjust volume to 100ml with distilled H_2O. Store stock (bulk substrate) at 5^{O}C and equilibrate substrate at 25^{O}C.

3.4 **Sample**

For codes 04003 and 04004: Weigh (accurately) between 5 and 10 mg enzyme and dissolve to a concentration of 1 mg / ml in ice-cold distilled water. Immediately before assay, dilute solution to yield 45-75 u/ml ice-cold distilled water. ($0,0075 \leq \Delta A_{260/min} \leq 0,0125$)

3.5 **Prepare DNase standard as follows:**

3.5.1 Use code 04003 as standard for codes 04003 and 04004

4. PROCEDURE:

λ: 260nm; Temp.: 25^{O}C; Light path: 10mm; Cuvette volume: 3,0ml.

Into a 10mm quartz cuvette pipette:

Substrate [3.3]	2,5ml
Equilibrate at 25^{O}C and monitor $\Delta A_{260/min}$	
Enzyme at zero time	0,5ml
	3,0ml

Record rate of increase in absorbance at 260nm for 3-6 minutes after initial time lag.

5. CALCULATION:

$$\text{Activity [u/mg]} = \frac{\Delta A_{260/min} \text{ X } 3 \text{ X dilution}}{0,001 \text{ X } 0,5 \text{ X mg enzyme / ml original solution}}$$

(ε = 0,001; cuvette volume = 3,0ml; enzyme volume = 0,5ml)

6. NOTE:

6.1 As the degree of polymerization of DNA in solution cannot be standardised, it is necessary to assay a standard DNase and to correct the unknown activity accordingly.

6.2 A DNase house standard is used for purposes of correcting the activity of the unknown sample. This standard is one which is standardised vs material of known activity.

7. BIBLIOGRAPHY:

Reference: Kunitz M.: (1950) J. Gen. Physiol 33 349.