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ASSAY / ANALYTICAL PROCEDURE
RIBONUCLEASE_KUNITZ

1. METHOD OF ASSAY:

Based on that of Kunitz in which the rate of decrease in absorbance caused by hydrolysis of yeast ribonucleic acid is determined at 300nm and 25^{O}C.

2. UNIT DEFINITION:

That amount of enzyme causing an absorbance decrease of initial absorbance A_o to final absorbance A_f per minute at 25^{O}C.

3. REAGENTS:

3.1 **0,1M Acetate Buffer pH5.0**

Dilute 5,7ml glacial acetic acid to 800ml with distilled H_2O, check pH5,0 with 5N NaOH and dilute to 1 000ml with distilled H_2O. Store at R/T.

3.2 **Substrate [0.05% Ribonucleic Acid]**

Dissolve 50mg highly polymerised and purified yeast RNA in 100ml buffer [3.1]. Store at R/T.

3.3 **Sample**

Dissolve 1mg enzyme/ml buffer [3.1]. Immediately before assay, dilute solution to yield 1,25 - 2,00 u/ml buffer. [ΔA/min 0,01 – 0,015]. Assay sample in triplicate.

4. PROCEDURE:

Set absorbance range of spectrophotometer on 0 – 0,5. Into 10mm quartz cuvettes pipette the following:

	Blank	Test
Substrate [3.2]	2,9 ml	2,9 ml
Equilibrate at 25^{O}C and monitor ΔA_{300}/min		
Enzyme [3.3] at zero time	-	0,1 ml
Buffer [3.1]	0,1 ml	
	3,0 ml	3,0 ml

Measure rate of decrease in absorbance at 300nm over initial 2,5 minutes only, by placing blank in test compartment and test in reference compartment of spectrophotometer to record an apparent “increase” in absorbance.

Now determine A_o-A_f factor by measuring absorbance of solutions (a) - (d) at 300nm in same 10mm quartz cell. Zero spectrophotometer against air and record absorbance of 2,9ml buffer (a), then add 0,1ml of 1mg enzyme/ml buffer and record absorbance (b). Rinse cell with1N HCl. Read absorbance of 2,9ml substrate (c), then add 0,1ml of 1mg enzyme/ml buffer, incubate at 25^{O}C for NLT 5 minutes until constant absorbance (d) is obtained. Rinse cell with 1N HCl.

5. CALCULATION:

$$A_o = c$$

$$A_f = \frac{d \times 3}{2,9} - (b - a)$$

$$\text{u/mg material} = \frac{\Delta A_{300} \times 3 \times \text{dilution}}{(A_o - A_f) \times 1 \times 0,1}$$

NOTE:

Ribonuclease adheres to glass, therefore rinse cell with 1N HCl.

6. BIBLIOGRAPHY:

Kunitz M,: (1946) J. Biol. Chem. 164 563.