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微球菌核酸酶检测方法

1 原理

Essentially that described by Heins et al. (1966) based upon the release of acid soluble oligonucleotides following nuclease digestion of DNA. One unit corresponds to a change in optical density of 1.0 at 260 nm at 37°C and pH 8.0 under the specified conditions.

2 试剂:

- 0.1 M Sodium borate, pH 8.8
- 0.01 M Calcium chloride
- DNA, 2.5 mg/ml: prepare by dissolving 25 mg Worthington Calf Thymus DNA in 10 ml 0.01 M NaCl. Allow to stand overnight at room temperature and then stir slowly to effect solution.
- 7% Perchloric acid
- 0.1% Bovine serum albumin

3 酶:

Dissolve at 1 mg/ml in reagent grade water. Dilute for assay to 0.001-0.002 mg/ml in 0.1% albumin.

4 操作规程:

Into each of 5 numbered test tubes pipette as follows:



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0.1 M Sodium borate, pH 8.8	0.1 ml
0.01 M CaCl ₂	0.05 ml
0.25% DNA	0.1 ml

Incubate in 37°C water bath for 6-7 minutes to achieve temperature equilibration. Prepare a minimum of four different dilutions of enzyme in the range of 0.001-0.0002 mg/ml. At timed intervals pipette 0.1 ml of appropriate dilution into respective tubes and replace in the water bath. To the fifth, which will serve as a blank, add 0.1 ml of 0.1% albumin. Incubate for 30 minutes. Stop the reaction at timed intervals by adding 0.5 ml 7% perchloric acid. Place the tubes in an ice bath for 10 minutes, then add 2.7 ml reagent grade water. Centrifuge for 15 minutes at highest speed on a clinical centrifuge. Withdraw clear supernatant and read A₂₆₀ versus the blank.

Note: For best results, sample should be diluted so that the final A₂₆₀ falls between 0.2-0.9.



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5 计算

$$\frac{\text{Units}}{\text{mg}} = \frac{(A_{260} - A_{260}^{\text{Blank}})}{\text{mg enzyme in reaction mixture}}$$