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磷酸二酯酶I 检测方法

1 原理

The assay is essentially that of Razell and Khorana (1959) where the reaction velocity is determined by an increase in absorbance at 400 nm resulting from the hydrolysis of p-nitrophenyl thymidine-5'-phosphate. One unit hydrolyzes one micromole of p-nitrophenyl thymidine-5'-phosphate per minute at pH 8.9 and 25°C under the specified conditions.

2 试剂：

- 0.11 M Tris· HCl buffer, pH 8.9, with 0.11 M NaCl and 15 mM $MgCl_2$ (Tris· Salts buffer)
- 5 mM p-nitrophenyl thymidine-5'-phosphate. Note: The purity of commercial preparations varies somewhat and should be considered in preparing this reagent.

3 酶：

Dissolve at 1 mg/ml in Tris*Salts buffer to obtain a rate of 0.02-0.04 ΔA/minute.

4 操作规程：

Set spectrophotometer at 400 nm and 25°C. Pipette into microcuvettes as

follows:

Tris·Salts buffer	0.9 ml
5 mM p-nitrophenyl thymidine-5'-phosphate	0.1 ml

Incubate cuvettes in spectrophotometer for 3-5 minutes to reach temperature equilibrium and establish blank rate, if any. Add 10 microliters of diluted enzyme and record increase in A_{400} for 3-5 minutes. The reaction remains linear until A_{400} reaches about 1.2. Calculate ΔA_{400}/minute from initial linear portion of absorbance curve.

5　计算

$$\frac{\text{Units}}{\text{mg}} = \frac{\frac{\Delta A_{400}}{\text{min}}}{16 \text{ x } \frac{\text{mg enzyme}}{\text{ml reaction mixture}}}$$

（where 16 is the extinction coefficient of p-nitrophenol under these conditions）