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Phosphatase, Alkaline (E. coli Enzyme)

Assay (E. coli Enzyme)

The assay is that of Garen and Levinthal (1960) in which the reaction velocity is determined by measuring an increase in absorbance at 410 nm resulting from the hydrolysis of p-nitrophenylphosphate to p-nitrophenol. One unit releases one micromole of p-nitrophenol per minute at 25°C, pH 8, under the specified conditions.

Reagents

- 1.5 M Tris· HCl buffer, pH 8.0
- 0.003 M p-nitrophenylphosphate (PNP). Care must be exercised to use an analytical grade and the correct molecular weight.

Enzyme

Dilute in reagent grade water to obtain a rate of 0.02-0.04 ΔA/minute.

Procedure

Adjust the spectrophotometer to 410 nm and 25°C.

Pipette into each cuvette as follows:

0.003 M PNP	1.0 ml
1.5 M Tris· HCl, pH 8.0	2.0 ml



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Mix well and incubate in the spectrophotometer for 4-5 minutes to achieve temperature equilibration and to establish blank rate, if any. Add 0.1 ml of diluted enzyme and record. Determine A₄₁₀ for 3-5 minutes from linear portion of the curve.

Calculation

$$\frac{\text{Units}}{\text{mg}} = \frac{\frac{\Delta A_{300}}{\text{min}} \times 1000}{3500 \times \frac{\text{mg enzyme}}{\text{ml reaction mixture}}},$$

where 3500 is the molar extinction coefficient of salicylic acid .