Assay (*E. coli* Enzyme)

Method: 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.

mg/ml=A_{278}×1.4

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

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 A_{410} for 3-5 minutes from linear portion of the curve.

Calculation

$$\text{Units/mg} = \frac{\Delta A_{410}/\text{min} \times 1000}{1.62 \times 104 \times \text{mg enzyme/ml reaction mixture}}$$