

Phosphatase, Alkaline (Calf Intestine Enzyme)

Assay (Calf Intestine Enzyme)

One unit hydrolyzes 1 umole of p-nitrophenol phosphate per minute at 37°C, pH 9.8.

Reagents

- 1.0 M Diethanolamine with 0.05 mM MgCl₂ buffer, pH 9.8: Dilute 12.4 gm diethanolamine (85%) with reagent grade water. Add 0.05 ml MgCl₂ solution (see below) and adjust the pH to 9.8 (at 37°C) with HCl. Adjust to 100 ml with reagent grade water.
- MgCl₂ solution: Dissolve 20.3 gm MgCl₂·6 H₂O in 100 ml reagent grade water.
- 0.67 M p-Nitrophenyl phosphate solution: Dissolve 250 mg p-nitrophenyl phosphate, Na salt in 1.0 ml reagent grade water.
- Diluent: 0.1 M TEA· HCl. Dissolve 1.86 gm TEA· HCl in reagent grade water, add 0.1 ml MgCl₂ solution and 0.1 ml 0.1 M ZnCl₂, adjust the pH to 7.6 with NaOH and adjust to 100 ml with reagent grade water.

Enzyme

Use diluent to obtain approximately 0.05 - 0.06 u/ml. Let stand 15-20 minutes at room temperature.

Procedure

Adjust the spectrophotometer to 405 nm and 37°C.

Pipette into cuvettes:

	Test	Blank
Buffer	3.00 ml	3.00 ml
4-nitrophenyl phosphate	0.050 ml	0.050 ml
Mix and incubate to achieve temperature equilibration.		
Diluent	-----	0.050 ml
Sample	0.050 ml	-----

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Mix. Measure the change in absorbance and calculate $\Delta A/\text{min}$ based on the linear range 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.

