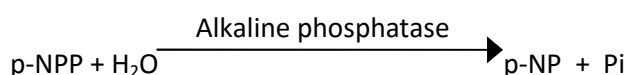


ASSAY / ANALYTICAL PROCEDURE
ALKALINE PHOSPHATASE

1. METHOD OF ASSAY:

Based on that of Bessey et al in which the rate of formation of the yellow colour of p-nitrophenol (p-NP) produced by hydrolysis of p-nitrophenylphosphate (p-NPP) in alkaline solution is measured spectrophotometrically at 405nm and 37°C.



2. UNIT DEFINITION:

That amount of enzyme which catalyses the liberation of 1 micromole p-nitrophenol per minute at 37°C.

3. REAGENTS:

3.1

0,3M 2-Amino-2 Methylpropane-1,3 Diol/0,002M MgCl₂ Buffer pH 10,25

Dissolve 3,16g AMPD [MM 105,14] in 80 ml distilled H₂O, adjust to pH10,25 (with 5M HCl), add 40,6mg MgCl₂.6H₂O [MM 203,3], dissolve, dilute to 100ml and recheck pH 10,25. Adjust with 1M NaOH or 5M HCl. Store diluent on ice, and store buffer at 37°C.

3.2

Substrate [0,4M p-Nitrophenyl Phosphate]

Dissolve 105mg Na₂p-NPP [MM 263,05] or 148,46 mg Na₂p-NPP.6H₂O [MM 371,15] / ml distilled H₂O. Store on ice.

3.3

Sample

For F/D product, dissolve 1mg enzyme/ml ice-cold buffer [3A]. Immediately before assay, dilute to yield ± 0,15 u/ml ice-cold buffer. [ΔA/min 0,09 – 0,12].

4. PROCEDURE:

λ: 405nm; Temp.: 37°C; cuvette volume: 3,0ml; Light Path: 10mm

Into a 10mm quartz cell, pipette:

Buffer [3.1] 2,8 ml

Substrate [3.2] 0,1 ml

Equilibrate at 37°C and monitor ΔA/min.

Enzyme [3.3] 0,1 ml

3,0 ml

Record rate of increase in absorbance at 405nm for ± 5 minutes.

5. CALCULATION:

$$\text{ACTIVITY [u/mg]} = \frac{\Delta A_{405/\text{min}} \times 3 \times \text{dilution}}{18,8 \times 0,1 \times \text{mg enzyme / ml original solution}}$$

[ε = 18,8 ; 3,0 = cuvette volume; 0,1 = enzyme volume]

6. REFERENCE: Bessey, O.A., Lowry O.H. and Brock M.J.:(1946) J.Biol. Chem. 164 321