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Product Information

Alkaline Phosphatase, Diethanolamine Detection Kit

Catalog Number **AP0100**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The Alkaline Phosphatase, Diethanolamine Detection Kit provides ready-to-use reagents for detecting the presence of alkaline phosphatase activity. This simple assay to detect alkaline phosphatase activity uses *p*-nitrophenyl phosphate (pNPP) as the substrate.

Alkaline phosphatase hydrolyzes pNPP to *p*-nitrophenol and inorganic phosphate. During incubation of the alkaline phosphatase sample and substrate at 37 °C, the reaction is followed by monitoring the increase in absorbance at 405 nm.

The assay can be performed using cuvettes or plates. The procedure provided is for detection using cuvettes with a 1 mL reaction volume.

Components

Each kit contains sufficient reagents for 100 assays (1 mL volume each).

Reaction Buffer: 500 mL
Catalog Number A5987
1.0 M Diethanolamine and 0.50 mM Magnesium Chloride, pH 9.8, at 37 °C

Phosphatase substrate: 1 g
Catalog Number P4744
p-Nitrophenyl Phosphate (pNPP)

Alkaline Phosphatase Control: 2 × 1 KU
Catalog Number P6774

Reagents and Equipment Required but Not Provided

- Pipettes and tips
- Ultrapure water
- Cuvettes or 96 well plates
- Containers for dilution
- Appropriate instrument to measure absorbance at 405 nm at a constant temperature of 37 °C.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

The Reaction Buffer is provided as a ready-to-use solution.

0.67 M pNPP Solution: Prepare 247 mg/mL of pPNPP (Catalog Number P4744) in ultrapure water. Prepare fresh and protect from light.

Alkaline Phosphatase Solution (enzyme control): Immediately before use, dilute the alkaline phosphatase (Catalog Number P6774) to ~0.15 units/mL in cold Reaction Buffer. Mix briefly to ensure the alkaline phosphatase is dissolved. Consult the Certificate of Analysis (CofA) of the specific lot for the concentration of the Alkaline Phosphatase Control.

Test Samples: Immediately before use, dilute samples to ~0.15 units/mL in cold Reaction Buffer. Mix briefly to ensure the alkaline phosphatase is dissolved.

Storage/Stability

The Reaction Buffer is stable for at least 2 years at 2–8 °C.

The Alkaline Phosphatase Control should be stored at 2–8 °C and is stable for at least 4 years.

The Phosphatase substrate should be stored at 2–8 °C.

For long term storage, the Alkaline Phosphatase Control and pNPP should be stored at –20 °C.

Procedure
Each researcher must determine the optimal conditions for the alkaline phosphatase specific to their application.

1. Pipette 980 µL of Reaction Buffer into one cuvette (blank).
2. Pipette 960 µL of Reaction Buffer into additional cuvettes (one for each test or enzyme control).
3. Add 20 µL of 0.67 M pNPP Solution to each cuvette (blank, test, and control).
4. Equilibrate the cuvettes to 37 °C.
5. Add 20 µL of the test sample to each test cuvette.
6. Add 20 µL of diluted Alkaline Phosphatase Solution to the enzyme control cuvette.
7. Immediately mix by inversion and record the increase in A_{405nm} for ~5 minutes. Obtain the maximum linear rate (ΔA_{405nm}/minute) for the test, blank, and control.

Results
Calculate the units/mL solution as follows:

$$\frac{(\Delta A_{405nm}/\text{min Test} - \Delta A_{405nm}/\text{min Blank})\ (df)\ (V_F)}{(18.5)\ (V_E)}$$

df = Dilution Factor
V_F = Volume (in mL) of assay
18.5 = Millimolar extinction coefficient of pNPP at 405 nm
V_E = Volume (in mL) of sample solution used

Unit Definition: One DEA unit will hydrolyze 1 µmole of *p*-nitrophenyl phosphate per minute at pH 9.8 at 37 °C. (One glycine unit is equivalent to ~3 DEA units.)

References
1. Walter, K., and Schutt, C., in *Methods of Enzymatic Analysis* (2nd ed.), Volume II (Hans-Ulrich Bergmeyer, ed.), Academic Press, Inc. (New York, NY), pp. 860-864 (1974).
2. Mössner, E., *et al.*, *Hoppe-Seyler's Z. Physiol. Chem.*, **361**, 543-549 (1980).

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