

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 one mL assays.

Reaction Buffer (Catalog Number A5987)	500 mL
1.0 M Diethanolamine and 0.50 mM Magnesium Chloride, pH 9.8, at 37 °C	
Phosphatase substrate (<i>p</i> -Nitrophenyl Phosphate, pNPP) (Catalog Number P4744)	1 g
Alkaline Phosphatase Control (Catalog Number P6774)	2 × 1 KU

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 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 Material 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 pNPP (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 CofA for concentration of 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

The 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 $^{\circ}\text{C}$.
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 $A_{405\text{nm}}$ for ~ 5 minutes. Obtain the maximum linear rate ($\Delta A_{405\text{nm}}/\text{minute}$) for the test, blank, and control.

Calculate the units/mg solid:

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

df = Dilution Factor

V_F = Volume (in milliliters of assay)

18.5 = Millimolar extinction coefficient of pNPP at 405 nm

V_E = Volume (in milliliters) of enzyme used

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

References

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

RBG,JJ,MAM 03/13-1