Product Information

Alkaline Phosphatase, Diethanolamine Detection Kit

Sufficient for 100 tests

AP0100

Product Description

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

Alkaline phosphatase hydrolyzes pNPP to *p*-nitrophenol and inorganic phosphate.[1,2] During incubation of the 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. Several dissertations have cited use of product AP0100 in their protocols.[3,4]

Components

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

Reaction Buffer (Component No. A5987), 1.0 M Diethanolamine and 0.50 mM Magnesium Chloride, pH 9.8, at 37 °C: 500 mL

Phosphatase substrate (Component No. P4744): *p*-Nitrophenyl Phosphate (pNPP): 1 g

Alkaline Phosphatase Control (Component No. P6774): 2 × 1 KU

Reagents and Equipment Required

(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

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.

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 the pNPP Phosphatase substrate should be stored at –20 °C.

Preparation Instructions

Reaction Buffer

This component is provided as a ready-to-use solution.

0.67 M pNPP Solution

- Prepare 247 mg/mL of pPNPP (Component No. P4744) in ultrapure water.
- Prepare fresh and protect from light.

Alkaline Phosphatase Solution (enzyme control)

- Immediately before use, dilute the alkaline phosphatase (Component No. 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.

Procedure

Each researcher must determine the optimal conditions for the alkaline phosphatase specific to her or his individual 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.
   - 7.1. Record the increase in A_{405nm} for ~5 minutes.
   - 7.2. Obtain the maximum linear rate (ΔA_{405nm}/minute) for the Test, Blank, and Control.

Results

Calculate the units/mL solution as follows:

$$\frac{[(\Delta A/\text{minute})^{Test} - (\Delta A/\text{minute})^{Blank})] * [(df)*(VF)]}{(18.5) * (VE)}$$

Where:

- A = A_{405nm}
- df = Dilution Factor
- VF = Volume (in mL) of assay
- 18.5 = Millimolar extinction coefficient of pNPP at 405 nm
- VE = 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.
- (Note: 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(4)**, 543-549 (1980).
3. Ozmadenci, Duygu, "Netrin-1 function in somatic cell reprogramming and pluripotency". Université de Lyon, Ph.D. dissertation, p. 14 (2017).
4. Wallis, Stephanie, "Investigating the pathogenesis of tau protein in stem cell models of neurodegenerative disease". University of Bristol, Ph.D. dissertation, p. 108 (2017).

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