EnzoLyte™ *p*NPP Alkaline Phosphatase Assay Kit

Colorimetric

Catalog #	71230
Unit Size	1 Kit
Kit Size	500 Assays (96-well) or 1250 Assays (384-well)

This kit is optimized to detect for alkaline phosphatase activity in biological samples or in alkaline phosphatase-conjugated secondary antibody in ELISA using *p*-Nitrophenyl phosphate (*p*NPP) as the colorimetric phosphatase substrate. It provides ample materials to perform 500 assays in a 96-well format or 1250 assays in a 384-well format. The kit has the following features:

- ***Convenient Format:*** Complete kit includes all the assay components.
- ***Optimized Performance:*** Optimal conditions for detecting alkaline phosphatase activity.
- ***Enhanced Value:*** Less expensive than the sum of individual components.
- ***High Speed:*** Minimal hands-on time.
- ***Assured Reliability:*** Detailed protocol and references are provided.

USA and Canada Ordering Information

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International Ordering Information

A list of international distributors is available at www.anaspec.com.

INTRODUCTION

The change in alkaline phosphatase level and activity is involved in a variety of physiological and pathological events such as bone development[1], bone-related diseases[2], gestation related diseases[3], inflammatory bowel disease[4], post-parathyroidectomy stage[5], and drug toxicity[6]. Alkaline phosphatase is also a popular enzyme conjugated to secondary antibody in ELISA.

The EnzoLyte™ *p*NPP Alkaline Phosphatase Assay Kit provides a convenient colorimetric assay for detecting alkaline phosphatase in biological samples and in alkaline phosphatase conjugated secondary antibody-based ELISA by using *p*NPP (*p*-Nitrophenyl phosphate) as a phosphatase substrate. Upon dephosphorylation by phosphatases, *p*NPP turns yellow and can be detected at absorbance=405 nm.

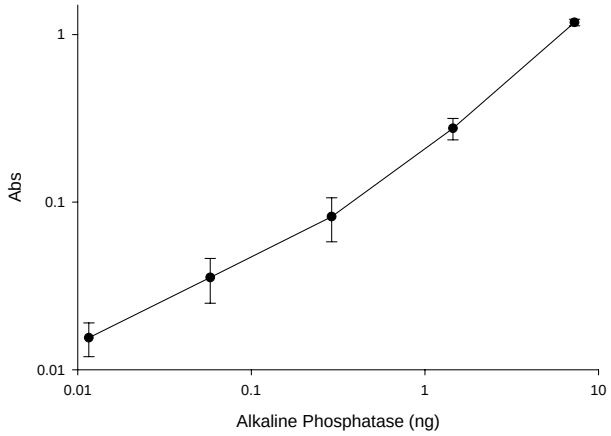

Figure 1. The *p*NPP can detect as low as 0.01 ng alkaline phosphatase with 10^3 linear range.

KIT COMPONENTS, STORAGE AND HANDLING

Note: Store component A at -20℃ and keep away from light. Store the rest of the kit components at 4℃.

Component A: *p*NPP, colorimetric alkaline phosphatase substrate (1 vial)

Component B: 2X Assay buffer (30 mL)

Component C: Stop solution (30 mL)

Component D: 10X Lysis buffer (50 mL)

Component E: Triton X-100 (500 µL)

Component G: Alkaline Phosphatase Standard, Calf Intestine (10 ng/µL, 50 µL, sterile)

OTHER MATERIALS REQUIRED (BUT NOT PROVIDED)

96-well or 384-well microplate: Clear microplate provides better signal for absorbance reading.
Absorbance plate reader: Capable of detecting absorbance at 405 nm.

STANDARD OPERATION PROTOCOL

Note 1: Warm all kit components to room temperature before starting the experiment.
Note 2: Choose Protocol A or B depending on your needs.

Protocol A Detect alkaline phosphatase activity in biological samples

Note: For preparation of biological samples containing alkaline phosphatase, please refer to Appendix I.

1. Prepare *p*NPP stock solution

- *p*NPP stock solution: Reconstitute by adding 250 µL of deionized water into the *p*NPP vial (component A). Mix the reagents well. Store this stock solution at -20°C and keep away from light.

2. Prepare *p*NPP reaction mixture

- Dilute the *p*NPP stock solution 1:100 with 2X assay buffer (component B). Prepare fresh reaction mixture for each experiment.

3. Alkaline phosphatase standard.

- Prepare alkaline phosphate dilution buffer: Dilute 10X lysis buffer (component D) to 1X with deionized water. Add bovine serum albumin to 1 mg/mL.
  Note: Bovine serum albumin is not included in the kit and should be provided by the investigator.
- Dilute alkaline phosphatase standard (10 ng/µL component G) to 0.2 ng/µL (1:50) in dilution buffer. Then make five-fold serial dilutions to get the concentration of 0.04, 0.008, 0.0016, 0.00032, 0.000064, 0.0000128, and 0 ng/µL of alkaline phosphatase solution.
  Note: Unused portion of diluted alkaline phosphatase solution should be discarded.

4. Detect alkaline phosphatase activity

- Add 50 µL/well (96-well plate) or 20 µL/well (384-well plate) of biological samples containing alkaline phosphatase.
- Set up alkaline phosphatase standard: Add 50 µL (96-well plate) or add 20 µL (384-well plate) serially diluted alkaline phosphatase standard solution from 0.2 to 0 ng/µL to the wells.
- Add 50 µL/well (96-well plate) or 20 µL/well (384-well plate) of *p*NPP reaction mixture. Mix the reagents by gently shaking the plate for 30 sec.
- Measure absorbance:
  For kinetic reading: Immediately start measuring absorbance at 405 nm continuously and record data every 5 min for 15 to 30 min.

  For end-point reading: Incubate reaction at the desired temperature for 15-30 min. (Optional) add 50 µL/well (96-well plate) or 20 µL/well (384-well plate) of stop solution (component C). Shake the plate on a plate shaker for 1 min before the reading. Measure absorbance at 405 nm.

Protocol B Detect alkaline phosphatase activity in ELISA

Note: For ELISA plate preparation, please refer to Appendix II.

1. Prepare stock solution (first time preparation only)

- *p*NPP stock solution: Reconstitute by adding 250 µL of deionized water into the *p*NPP vial (component A). Mix the reagents well. Store this stock solution at -20°C, keep away from light.

2. Prepare *p*NPP reaction mixture

- Dilute 2X assay buffer (component B) to 1X in deionized water.
- Dilute *p*NPP stock solution (component A) 1:200 with 1X assay buffer. Keep the reaction mixture from light. Prepare fresh reaction mixture for each experiment.

3. Detect alkaline phosphatase activity

- Add 100 µL/well (96-well plate) or 20 µL/well (384-well plate) of *p*NPP reaction mixture. Mix the reagents by gently shaking the plate for 30 sec.
- Measure absorbance:
  For kinetic reading: Immediately start measuring absorbance at 405 nm continuously and record data every 5 min for 15 to 30 min.

  For end-point reading: Incubate reaction at the desired temperature for 15-30 min. (Optional) add 50 µL/well (96-well plate) or 20 µL/well (384-well plate) of stop solution (component C). Measure absorbance at 405 nm.

Appendix I

Prepare cell extract for alkaline phosphatase

- Prepare 1X lysis buffer by adding 1 mL of 10X lysis buffer (component D) to 9 mL of deionized-water.
- Wash cells with 1X lysis buffer twice gently.
- Add 20 µL of Triton X-100 (component E) to 10 mL of 1X lysis buffer, mix well. Add an appropriate amount of 1X lysis buffer to cells or cell pellet. Scrape off the adherent cells or resuspend the cell pellet. Collect the cell suspension in a microcentrifuge tube.
- Incubate the cell suspension at 4°C for 10 min under agitation.
- Centrifuge the cell suspension at 2500 X g for 10 min at 4°C.
- Collect the supernatant for alkaline phosphatase assay.

Prepare tissue extract for alkaline phosphatase

- Prepare 1X lysis buffer by adding 20 µL of Trition-X 100 (component E), 1 mL of 10X lysis buffer (component D) to 9 mL of deionized- water.
- Homogenize tissue in 1X lysis buffer, and then centrifuge for 15 min at 10000x g at 4°C. Collect the supernatant for the alkaline phosphatase assay.

Appendix. General ELISA protocol

1. Required buffers:

1. Coating buffer: 1.59 g of Na_2CO_3 and 2.93 g of $NaHCO_3$ in1 L of deionized H_2O. The pH would be 9.6 without adjustment.
2. Tris-buffered saline (TBS): 8.76 g of NaCl, 12.1 g of Tris in 800 ml of deionized H_2O. Adjust the pH to 7.4 with HCl. Add H_2O to 1L.
3. Blocking buffer: add 10 g of bovine serum albumin (BSA) and 0.2 mL of Tween®-20 into 1 L of TBS.
4. EIA buffer: add 1 g of bovine serum albumin (BSA) and Tween®-20 into 1 L of TBS.
5. Wash buffer: add 0.2 mL of Tween®-20 into 1 L of TBS.

2. ELISA procedures:

1. Coating: add 100 µL of peptide-conjugate (PP-BSA) to each well of the 96-well plate at a concentration of 10 µg/mL in coating buffer. Seal the plate with plate sealer and incubate at 4°C overnight.
2. Washing: discard the solution and wash the plate with 300 µL of wash buffer per well three to five times. Soak the plate during the last wash step for 5 minutes. Pad dry on paper towel.
3. Blocking: add 200 µL of blocking buffer and incubate 1h at room temperature.
4. Washing: repeat Step 2.
5. Add the primary antibody: dilute anti-peptide antibody in EIA buffer to appropriate concentration. Add 100µL of the diluted antibody to each well and incubate at room temperature for 1h on a plate shaker.
6. Washing: repeat Step 2.
7. Add the secondary antibody: dilute alkaline phosphatase conjugated secondary antibody in EIA buffer to the appropriate concentration (1:2000 dilution or 50 ng per well is recommended. Alkaline phosphatase-conjugated goat anti rabbit IgG (component F) is provided in this kit). Add 100 µL of diluted secondary antibody to each well and incubate at room temperature for 1h on a plate shaker.
8. Washing: repeat Step 2.
9. Detection by substrate: The plate is now ready for the *p*NPP detection (refer to Standard Operation Protocol).

References

1. N. Kotobuki, M. Hirose, H. Funaoka, H. Ohgushi, *Cell Transplant.* 13, 377-383 (2004).
2. M. H. Wyckoff et al., *J.Clin.Endocrinol.Metab* (2004).
3. A. Boronkai et al., *J.Clin.Pathol.* 58, 72-76 (2005).
4. d. M. Sanchez et al., *Biochem.Pharmacol.* 68, 2317-2326 (2004).
5. L. F. Morrone, M. Tampoia, N. Pansini, L. Gesualdo, *Ann.Ital.Med.Int.* 19, 189-192 (2004).
6. P. Papaldo et al., *Cancer Invest* 22, 650-653 (2004).