SensoLyte™ *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 alkaline phosphatase activity in biological samples or in ELISA with alkaline phosphatase-conjugated secondary antibody 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

AnaSpec Corporate Headquarter
2149 O'Toole Ave.
San Jose, CA 95131
Toll-Free: 800-452-5530
Tel: 408-452-5055
Fax: 408-452-5059
Email: service@anaspec.com
Website: www.anaspec.com

International Ordering Information

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

Version 1.3

コスモ・バイオ株式会社

INTRODUCTION

The change in alkaline phosphatase level and activity is implicated 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 SensoLyte™ *p*NPP Alkaline Phosphatase Assay Kit provides a convenient colorimetric assay for detecting alkaline phosphatase in biological samples and in ELISA with alkaline phosphatase conjugated secondary antibody 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.

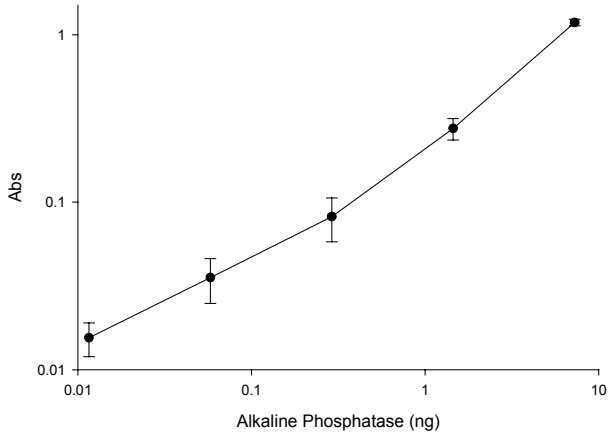

Figure 1. *p*NPP can detect as low as 0.01 ng alkaline phosphatase with 10^3 linear range.

KIT COMPONENTS, STORAGE AND HANDLING

Note: Store all 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 F: Alkaline Phosphatase Standard, Calf Intestine (10 µg/mL, 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.

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. Detecting 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 the substrate by adding 232 µL of deionized water into the *p*NPP vial (Component A). Mix the reagents thoroughly. The stock solution is good for 3-4 weeks if stored at -20°C.

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 µg/mL - Component F) to 0.2 µg/mL (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 µg/mL 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 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 and continuously 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. Detecting 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 the substrate by adding 232 µL of deionized water into the *p*NPP vial (Component A). Mix the reagents thoroughly. The stock solution is good for 3-4 weeks if stored at -20°C.

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 and continuously 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.
- Gently wash cells twice with 1X lysis buffer.
- 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 Triton-X 100 (Component E) and 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 II. 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. pH is ~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 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:

1. Coating: Add 100 µL of peptide-conjugate (PP-BSA) or protein 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 or anti-protein 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 (1:1,000 to 1:10,000 dilution). 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 Protocol B).

References

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