SensoLyte® *p*NPP Alkaline Phosphatase Assay Kit **Colorimetric**

Catalog #	72146
Kit Size	500 Assays (96-well plate)

- ***Optimized Performance:*** This kit is optimized to detect alkaline phosphatase activity.
- ***Enhanced Value:*** It provides ample reagents to perform 500 assays in a 96-well format.
- ***High Speed:*** The entire process can be completed in one hour.
- ***Assured Reliability:*** Detailed protocol and references are provided

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	*p*NPP, colorimetric alkaline phosphatase substrate	25 mL
Component B	10X Assay buffer	50 mL
Component C	Stop solution	25 mL
Component D	Triton-X-100	500 µL
Component E	Alkaline Phosphatase Standard, Calf Intestine	10 µg/mL, 50 µL

Other Materials Required (but not provided)

- 96-well microplate: Clear microplate provides better signal to noise ratio
- Absorbance plate reader: Capable of detecting absorbance at 405 nm.

Storage and Handling

- Store all components at 4°C.
- Keep Component A away from light.

Introduction

Changes in alkaline phosphatase level and activity are 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 widely used in ELISA for conjugation with secondary antibody and as a reporter for gene expression studies.

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 or streptavidin by using colorimetric *p*NPP (*p*-Nitrophenyl phosphate) phosphatase substrate. Upon dephosphorylation, *p*NPP turns yellow and can be detected at absorbance=405 nm. The assay can detect as low as 0.2 ng/mL active alkaline phosphatase.

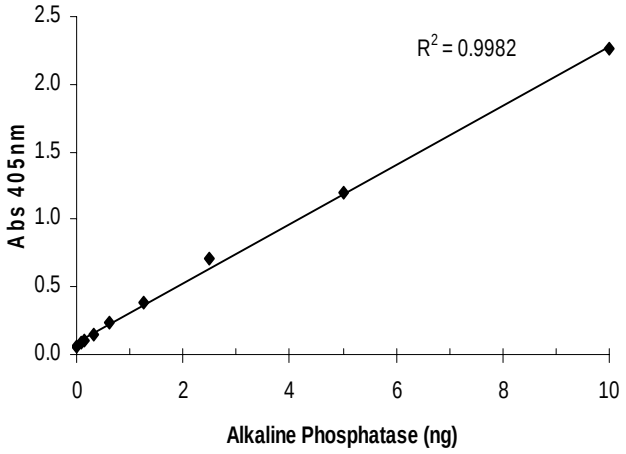

Figure 1. Detection of alkaline phosphatase with the SensoLyte® *p*NPP Alkaline Phosphatase Assay Kit.
The alkaline phosphatase enzyme at each dilution was mixed with *p*NPP substrate, and then incubated at room temperature for 1 hr. Absorbance was recorded at 405 nm (Ultra Microplate Reader EL808, Bio-Tek Instruments, Inc).

Protocol

Note 1: Warm all kit components to room temperature before starting the experiment.

Note 2: Please use Protocol A or B based on your needs.

Protocol A. Detecting alkaline phosphatase activity in biological samples

1. Prepare working solutions.

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

1.1 *p*NPP alkaline phosphatase substrate (Component A): Ready to use.

1.2 Prepare alkaline phosphatase dilution buffer: Dilute 10X assay buffer (Component B) to 1X Assay buffer with deionized water.

1.2 Alkaline phosphatase standard: Dilute alkaline phosphatase standard (10 µg/mL - Component E) to 0.2 µg/mL (1:50) in dilution buffer. Then make two-fold serial dilutions to get the concentration of 100, 50, 25, 12.5, 6.2, 3.1, and 0 ng/mL of alkaline phosphatase solution.

Note: Unused portion of diluted alkaline phosphatase solution should be discarded.

2. Set up the enzymatic reaction.

2.1 Add 50 µL/well of biological samples containing alkaline phosphatase. Use alkaline phosphatase dilution buffer (Step 1.2) to dilute samples.

2.2 Set up alkaline phosphatase standard (optional): Add 50 µL of serially diluted alkaline phosphatase standard solution from 200 to 0 ng/mL to the wells. The final amounts of alkaline phosphatase standard are 10, 5, 2.5, 1.2, 0.6, 0.3, 0.15, and 0 nanogram/well.

3. Detect alkaline phosphatase activity.

3.1 Add 50 µL of *p*NPP substrate solution into each well. Mix the reagents by gently shaking the plate for 30 sec.

3.2 Measure absorbance:

- For kinetic reading: Immediately start measuring absorbance at 405 nm and continuously record data every 5 min for 30 to 60 min.
- For end-point reading: Incubate reaction at the desired temperature for 30-60 min. Optional: Add 50 µL of Stop Solution (Component C) into each well. 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

1. Prepare working solutions.

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

1.1 1X assay buffer: Dilute 10X assay buffer (Component B) to 1X assay buffer with deionized water.

1.2 *p*NPP alkaline phosphatase substrate working solution: Add 5 mL of *p*NPP substrate solution (Component A) to 5 mL of 1X assay buffer. This amount of substrate is enough for one 96-well plate.

Note: Prepare fresh working solutions for each experiment.

2. Detect alkaline phosphatase activity.

2.2 Add 100 µL of *p*NPP substrate working solution from step 1.2 into each well. Mix the reagents by gently shaking the plate for 30 sec.

2.3 Measure absorbance: Incubate reaction at the desired temperature for 30-60 min. Add 50 µL of Stop Solution (Component C) into each well. Measure absorbance at 405 nm.

Appendix I

Prepare cell extract for alkaline phosphatase

- Prepare 1X assay buffer by adding 1 mL of 10X assay buffer (Component B) to 9 mL of deionized water.
- Gently wash cells twice with 1X assay buffer.
- Add 20 µL of Triton X-100 (Component D) to 10 mL of 1X assay buffer, mix well. Add an appropriate amount of 1X assay 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 assay buffer by adding 20 µL of Triton-X 100 (Component D) and 1 mL of 10X assay buffer (Component B) to 9 mL of deionized water.
- Homogenize tissue in 1X assay buffer, and then centrifuge for 15 min at 10,000x 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 $NaHCO_3$ in 1 L of deionized H_2O. The 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 BSA and 0.2 mL of Tween®-20 into 1 L of TBS.
4. EIA buffer: Add 1 g of BSA and 0.2 mL Tween®-20 into 1 L of TBS.
5. Wash buffer: Add 0.2 mL of Tween®-20 into 1 L of TBS.

2. Required ELISA microplate:

Use clear, high-binding ELISA plates for better signal to noise ratio.

3. ELISA:

1. Coating: Add 100 µL of capture antibody to each well of the 96-well plate at a concentration of 2-10 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 200 µL of wash buffer per well three to five times. Soak the plate during the last wash step for 5 min. 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 sample: Dilute sample to be tested in EIA buffer to an appropriate concentration. Add 100 µL of the diluted sample to each well and incubate at room temperature for 1h on a plate shaker.
6. Washing: Repeat Step 2.
7. Add detection antibody: Dilute alkaline phosphatase conjugated detection antibody in EIA buffer to the appropriate concentration (1:500 to 1:5000 dilution). Add 100 µL of diluted antibody to each well and incubate at room temperature for 1h on a plate shaker.
8. Washing: Repeat Step 2.
9. Detection by substrate: Plate is now ready for *p*NPP detection (refer to Protocol B).

References

1. Kotobuki, N. et al. *Cell Transplant.* 13, 377 (2004).
2. Wyckoff, M.H. et al. *J. Clin. Endocrinol. Metab.* 90, 1233 (2004).
3. Boronkai, A. et al. *J. Clin. Pathol.* 58, 72 (2005).
4. Sanchez, M. et al. *Biochem. Pharmacol.* 68, 2317 (2004).
5. Morrone, L.F. et al. *Ann. Ital. Med. Int.* 19, 189 (2004).
6. Papaldo, P. et al. *Cancer Invest.* 22, 650 (2004).