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

| Catalog # | 71232-M |
|---|---|
| **Kit Size** | 500 Assays |

- ***Convenient Format:*** Complete kit including all the assay components.
- ***Optimized Performance:*** Optimal conditions for AP-labeled secondary antibody detection.
- ***Enhanced Value:*** Less expensive than the sum of individual components.
- ***High Speed:*** Minimal hands-on time.
- ***Assured Reliability:*** Detailed protocol is provided.

## Kit Components, Storage and Handling

| Component | Description | Quantity |
|---|---|---|
| Component A | *p*NPP, colorimetric alkaline phosphatase substrate | 1 vial |
| Component B | Assay buffer | 60 mL |
| Component C | Stop solution | 30 mL |
| Component D | 10X Wash buffer | 60 mL |
| Component E | Alkaline phosphatase-conjugated goat anti-mouse IgG | 50 µL |

### Other Materials Required (but not provided)

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

### Storage and Handling

- Store Component A at -20°C, others at 4°C.

## Introduction

Alkaline phosphatase (AP) is widely used in ELISA for conjugation with secondary antibody or streptavidin. The SensoLyte® *p*NPP Alkaline Phosphatase Assay Kit is designed to detect alkaline phosphatase conjugates in ELISA. *p*NPP is a colorimetric substrate for alkaline phosphatase and the absorbance can be monitored at 405 nm.

## Protocol

Note 1: Prepare ELISA assay plate according to standard ELISA procedures (refer to **Appendix**). Alkaline phosphatase conjugated goat anti-mouse IgG (Component E) is provided in the kit.
Note 2: Warm all the kit components to room temperature when the ELISA plate is ready for detection.

**1. Prepare stock solution (for first time preparation only).**

1.1 *p*NPP stock solution: Add 250 µL of deionized water into the *p*NPP vial (Component A). Mix the reagent well. The stock solution is good for 3-4 weeks if stored at -20°C.

**2. Prepare *p*NPP reaction mixture.**

2.1 Dilute *p*NPP stock solution 1:200 in assay buffer (Component B). Keep the reaction mixture away from light.

2.2 Optional: If phosphate-buffered saline was used in the ELISA procedures, the microplate must be washed with wash buffer provided in the kit:

- Dilute 10X wash buffer (Component D) to 1X in deionized water.
- Wash microplate with 200µL 1X wash buffer for three times, then pad dry on paper towels. For better sensitivity, we recommend using the buffer sets described in **Appendix**.

**3. Detect alkaline phosphatase activity.**

3.1 Add 100µL/well of *p*NPP reaction mixture in a 96-well plate.

3.2 Incubate the reaction for 15 to 30 min, keep away from light.

Note: The reaction can be stopped by adding 50µL/well of stop solution (Component C). The signal is stable for at least 45 minutes.

3.3 Shake the plate on a plate shaker for 1 min before the reading. Read plate using an absorbance microplate reader at 405 nm.

### Appendix. 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 $H_2O$. 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 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 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 mouse detection antibody in EIA buffer to the appropriate concentration. 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. Add secondary antibody: Dilute alkaline phosphatase-conjugated goat anti-mouse antibody (Component E) in EIA buffer to the appropriate concentration (1:1,000 to 1:10,000 dilution). Add 100 µL of diluted antibody to each well and incubate at room temperature for 1h on a plate shaker.
10. Washing: Repeat Step 2.
11. Detection by substrate: Plate is now ready for *p*NPP detection (proceed to Protocol).