SensoLyte™ *p*NPP Alkaline Phosphatase ELISA Assay Kit

Colorimetric

Catalog #	71232-R
Unit Size	1 Kit
Kit Size	500 Assays

This kit is optimized to detect alkaline phosphatase-labeled secondary antibody or streptavidin using *p*-Nitrophenyl phosphate (*p*NPP) as the colorimetric phosphatase substrate. The alkaline phosphatase conjugated goat anti-rabbit IgG is included in the kit. It provides ample material to perform 500 ELISA assays in a 96-well format. The protocol can readily be modified to run assays in a 384-well format. The kit has the following features:

- ***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 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 SensoLyte™ *p*NPP Alkaline Phosphatase Assay Kit is used in ELISA which alkaline phosphatase (AP) conjugates, AP-antibody or AP-streptavidin, are served as the secondary detection system. *p*NPP is a colorimetric substrate for alkaline phosphatase and the absorbance can be monitored at the absorbance of 405 nm.

KIT COMPONENTS, STORAGE AND HANDLING

Note: Store all components at 4℃.

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-rabbit IgG (50 µL)

OTHER MATERIALS REQUIRED (BUT NOT PROVIDED)
96-well microplate: Clear ELISA microplate provides better signal to noise value for absorbance reading.
Absorbance microplate reader: Capable of detecting absorbance at 405 nm.

STANDARD OPERATION PROTOCOL

*Note 1: Prepare ELISA assay plate according to standard ELISA procedures (refer to **Appendix**). Alkaline phosphatase-conjugated goat anti-rabbit IgG (component F) 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):

- *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:

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

3. (Optional) if phosphate-buffered saline was used in ELISA procedures, the microplate must be washed with 1X wash buffer:

- 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**.

4. Detect alkaline phosphatase activity:

- Add 100µL/well of *p*NPP reaction mixture in a 96-well plate.
- 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.
- 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 $NaHCO_3$ 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 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/well of protein to the 96-well ELISA plate at a concentration of 2-10 µg/mL in coating buffer. Incubate the plate at 4°C overnight.
2. Blocking: Discard the solution. Add 200 µL of blocking buffer and incubate 30 min to 1 h at room temperature. Discard the blocking reagent and dry the plate under vacuum. You can store the plate at 4°C for future use.
3. Washing: 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 minutes. Pad dry on paper towel.
4. Add sample: Add 50-100 µL/well of sample to be tested and incubate at room temperature for 1 or more hours on a plate shaker. The sample can be diluted in EIA buffer or other appropriate buffer before adding to the plate.
5. Washing: Repeat Step 3.
6. Add enzyme-conjugated secondary antibody: Dilute alkaline phosphatase conjugated secondary antibody in EIA buffer to an appropriate concentration (1:1,000 –1:10,000 dilution). Alkaline phosphatase-conjugated goat anti rabbit IgG (component F) is provided in the kit. Add 100 µL/well of diluted secondary antibody and incubate at room temperature for 1 h on a plate shaker.
7. Washing: Repeat Step 3.
8. Detect by adding substrate: The plate is now ready for the *p*NPP detection (refer to Standard Operation Protocol).