

SensoLyte® pNPP Alkaline Phosphatase

ELISA Assay Kit *Colorimetric*

Catalog #	72147-G
Kit Size	500 Assays (96-well plate)

• *Optimized Performance:* This kit is optimized to detect alkaline phosphatase labeled conjugates in ELISA.

- Enhanced Value: It provides ample reagents to perform 500 assays in a 96-well format.
- Assured Reliability: Detailed protocol is provided

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	<i>p</i> NPP, colorimetric alkaline phosphatase substrate	25 mL
Component B Assay buffer		25 mL
Component C	10X Wash buffer	50 mL
Component D	Stop solution	25 mL
Component E	Alkaline phosphatase-conjugated rabbit anti- goat IgG	50 µL

Other Materials Required (but not provided)

- <u>96-well microplate</u>: Clear ELISA microplates provide 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

Alkaline phosphatase is widely used in ELISA for conjugation with secondary antibody or streptavidin. The SensoLyte[®] *pNPP* Alkaline Phosphatase ELISA Assay Kit is optimized to detect alkaline phosphatase (AP) conjugated secondary antibody or streptavidin in ELISA. *pNPP* is a colorimetric substrate for alkaline phosphatase and the absorbance can be monitored at 405 nm. The kit provides ample material to perform 500 assays in a 96-well format. The alkaline phosphatase conjugated rabbit anti-goat IgG is included in the kit. The protocol can readily be modified to run assays in a 384-well format.

Protocol

1. Prepare working solutions.

<u>Note 1</u>: Prepare an ELISA assay plate according to standard ELISA procedures (see Appendix). Alkaline phosphatase-conjugated rabbit anti-goat IgG (Component E) is provided in the kit.

Note 2: Bring all kit components to room temperature when the ELISA plate is ready for detection.

<u>1.1</u> Prepare pNPP working solution: Add 5 mL of pNPP substrate solution (Component A) to 5 mL of assay buffer (Component B). This amount of substrate is enough for one 96-well plate.

Note: Prepare fresh dilution for each experiment.

- <u>1.2</u> <u>Optional</u>: If phosphate-buffered saline was used in the ELISA procedures, the microplate must be washed with wash buffer provided in the kit:
 - Add 10 mL of 10 X wash buffer (Component C) to 90 mL deionized water to get 1X wash buffer.
 - Wash microplate with 200 µL 1X wash buffer for three times, then pad dry on paper towels. For better sensitivity, we recommend using the buffer sets described in Appendix.

2. Detect alkaline phosphatase activity.

- <u>2.1</u> Add 100 μ L of prepared *p*NPP working solution into each well.
- 2.2 Incubate the reaction for 30 to 60 min.
- 2.3 Add 50 µL of Stop Solution (Component D) into each well. After the stop solution is added, the signal is stable for at least 45 min.
- 2.4 Measure absorbance at 405 nm.

Appendix: General ELISA protocol

<u>1.</u> Required buffers:

- 1. Coating buffer: 1.59 g of Na₂CO₃ and 2.93 g of NaHCO₃ in 1 L of deionized H₂O. 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 1 L.
- 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 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. <u>Required ELISA microplate:</u>

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

<u>3. ELISA</u>:

- 1. <u>Coating</u>: Add 100 μ L of capture antibody to each well of the 96-well plate at a concentration of 2-10 μ g/mL in coating buffer. Seal the plate with plate sealer and incubate at 4°C overnight.
- 2. <u>Washing</u>: 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. <u>Blocking</u>: Add 200 µL of blocking buffer and incubate 1h at room temperature.
- 4. <u>Washing</u>: Repeat Step 2.
- 5. <u>Add sample</u>: Dilute sample to be tested in EIA buffer to an appropriate concentration. Add 100 μ L of the diluted sample to each well and incubate at room temperature for 1h on a plate shaker.
- 6. <u>Washing</u>: Repeat Step 2.
- <u>Add detection antibody</u>: Dilute goat 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. <u>Washing</u>: Repeat Step 2.
- <u>Add secondary antibody</u>: Dilute alkaline phosphatase-conjugated rabbit anti-goat 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 pNPP detection (see Protocol)