SensoLyte® Luminescent Alkaline Phosphatase Assay Kit **Luminometric**

Revision Number: 1.2	*Last updated: July 2021*
Catalog #	**AS-72122**
Kit Size	500 Assays (96-well plate)

- ***Optimized Performance:*** This kit is optimized to detect alkaline phosphatase activity.
- ***Enhanced Value:*** It provides enough reagents to perform 500 assays in a 96-well format.
- ***High Speed:*** The entire process can be completed in 30 minutes.
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	Chemiluminescent substrate	25 mL
Component B	10X Assay buffer	50 mL
Component C	Alkaline Phosphatase Standard, Calf Intestine	10 µg/mL, 50 µL
Component D	Triton X-100	500 µL
Component E	Stop Solution	30 mL

Other Materials Required (But not Provided)

- 96-well microplate: White microplates provide better signal to noise ratio for luminescence reading.
- Luminescent microplate reader

Storage and Handling

- Store all kit components at 4°C.
- Protect Component A 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® Luminescent Alkaline Phosphatase Assay Kit detects alkaline phosphatase activity in biological samples, in cell culture media and in ELISA with alkaline phosphatase-conjugated secondary antibody or streptavidin by using a chemiluminescent substrate. The assay can detect femtogram level of alkaline phosphatase and has 10^4 linear range.

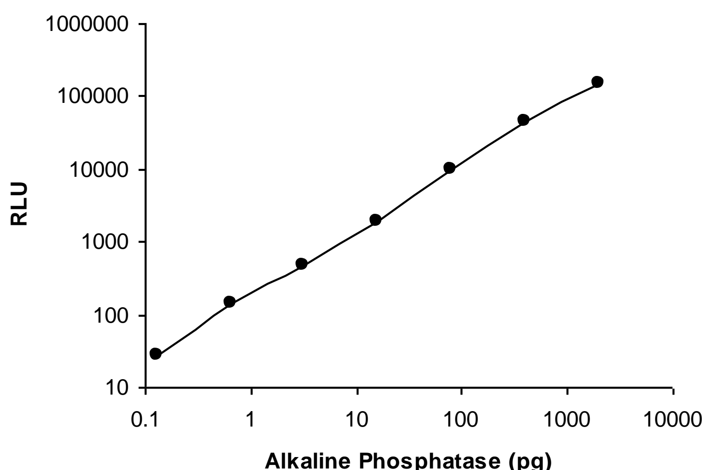

Figure 1. Detection of alkaline phosphatase with the SensoLyte® Luminescent Alkaline Phosphatase Assay Kit. The detection limit can reach as low as 0.1 pg of enzyme.

Protocol

Note 1: Bring all kit components, except Component C (CIAP), to room temperature before starting the experiment.
Note 2: Keep the enzyme (Component C) on ice.
Note 3: Please use Protocol A or B based 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 working solutions.

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

1.2 Prepare dilutions of alkaline phosphatase: Dilute alkaline phosphatase standard (10 µg/mL - Component C) to 0.04 µg/mL (1:250) in dilution buffer. Then make five-fold serial dilutions to get the concentration of 8000, 1600, 320, 64, 12.8, 2.56 and 0 pg/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 of biological samples containing alkaline phosphatase to the wells.

2.2 Set up alkaline phosphatase standard: Add 50 µL serially diluted alkaline phosphatase reference solutions to the wells. The final amounts of alkaline phosphatase standard are 2000, 400, 80, 16, 3.2, 0.64, 0.128, and 0 picogram/well.

2.3 Simultaneously establish the following control wells, as deemed necessary:

- Negative control contains 50 µL of biological sample without alkaline phosphatase.
- Substrate control contains 50 µL of alkaline phosphatase dilution buffer.

3. Run the enzymatic reaction.

3.1 Add 50 µL/well of chemiluminescent substrate (Component A) into each well. Mix the reagents completely by shaking the plate gently for 30 sec.

3.2 Measure luminescent signal:

- For kinetic reading: Immediately start measuring luminescence intensity and continuously record data every 5 min. for 15 to 30 min.
- For end-point reading: Incubate the reaction for 15 to 30 min. Keep plate from direct light. Optional: Add 50 µL of Stop Solution (Component E) to each well. Measure luminescence intensity.

3.3. Data analysis: The luminescence reading from the substrate control well is the background luminescence. This background reading should be subtracted from the readings of the other wells. The luminescence readings are expressed in relative luminescence units (RLU).

Protocol B. Detecting alkaline phosphatase activity in ELISA

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

1. Prepare working solutions.

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

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

2. Detect alkaline phosphatase activity

2.1. Add 100 µl/well of diluted chemiluminescent alkaline phosphatase substrate into each well. Shake the plate gently for 30 sec.

2.2. Measure luminescence signal: Incubate reaction for 15 to 30 min and keep plate away from light. Optional: Add 50 µL/well of Stop Solution (Component E). Measure luminescence intensity.

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.
- Prepare 1X lysis buffer by adding 20 μL of Triton X-100 (Component D) to 10 mL of 1X assay 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 2,500 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 D) and 1 mL of 10X assay buffer (Component B) to 9 mL of deionized water.
- Homogenize tissue in 1X lysis buffer, and then centrifuge for 15 min at 10,000x g at 4°C. Collect the supernatant for 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 white or black 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 the luminescence detection (refer to the Protocol B).

Reference

1. Kotobuki, N et al. *Cell Transplant.* 13, 377 (2004).
2. Wyckoff, MH. 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, LF. et al. *Ann. Ital. Med. Int.* 19, 189 (2004).
6. Papaldo, P et al. *Cancer Invest.* 22, 650 (2004).