SensoLyte® FDP Alkaline Phosphatase Assay Kit
Fluorimetric

Catalog #	AS-71109
Kit Size	500 Assays (96-well) or 1250 Assays (384-well)

- ***Convenient Format:*** Complete kit includes all the assay components.
- ***Optimized Performance:*** Optimal conditions for detecting alkaline phosphatase activity.
- ***Enhanced Value:*** Less expensive than the sum of individual components.
- ***High Speed:*** Minimal hands-on time.
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	FDP, fluorogenic alkaline phosphatase substrate	1 vial
Component B	2X Assay buffer	30 mL
Component C	Stop solution	30 mL
Component D	10X Lysis buffer	50 mL
Component E	Triton X-100	500 µL
Component F	DMSO	500 µL
Component G	Alkaline Phosphatase Standard, Calf Intestine	10 µg/mL, 50 µL, sterile

Other Materials Required (but not provided)

- 96-well or 384-well microplate: Black microplate provides better signal to noise ratio for fluorescence reading.
- Fluorescence microplate reader: Capable of detecting emission at 528±20 nm with excitation at 485±20 nm.

Storage and Handling

- Store all components at –20°C.

ANASPEC INC.,
34801 Campus Drive, Fremont, CA 94555 • Tel 800 452 5530 • Fax 510 791 9572
www.anaspec.com

Introduction

The change in alkaline phosphatase level and activity is 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.

The SensoLyte® FDP Alkaline Phosphatase Assay Kit provides a convenient fluorogenic assay for detection of alkaline phosphatase activity in biological samples and in ELISA with alkaline phosphatase-conjugated secondary antibody by using FDP (3,6-fluorescein diphosphate) as a fluorogenic phosphatase substrate. Fluorescein, the final hydrolytic product of FDP, has a very high emission quantum yield. As a result, the assay is more sensitive than its colorimetric counterparts. The assay can detect 0.5 pg of alkaline phosphatase and has 10^3 linear range (Figure 1). The signal of fluorescein can be easily read by a fluorescence plate reader at Ex/Em=485±20/528±20 nm.

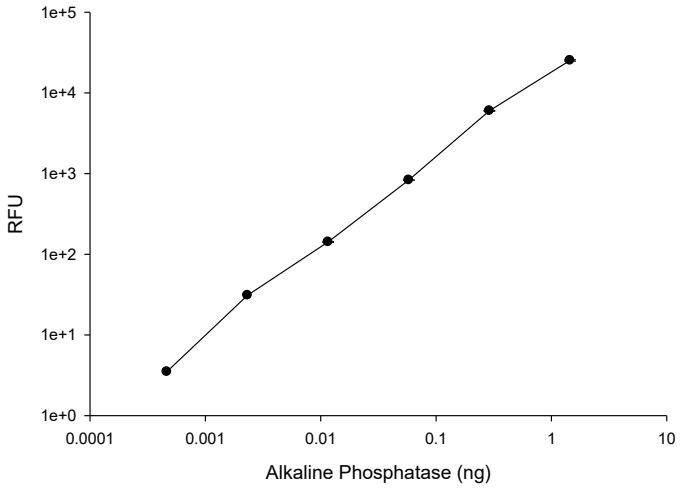

Figure 1. FDP can detect as low as 0.5 pg of alkaline phosphatase with 10^3 linear range.

Protocol

Note 1: Warm all kit components until thawed to room temperature before starting the experiments.
Note 2: Choose Protocol A or B depending 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 FDP stock solution.

1.1 FDP stock solution: Reconstitute the substrate by adding 250 µL of DMSO (Component F) into the FDP vial (Component A). Mix the reagents thoroughly. The stock solution is good for 3-4 months if stored at -20°C.

2. Prepare FDP reaction mixture.

2.1 Dilute FDP stock solution (Component A) 1:100 with 2X assay buffer (Component B). Keep the reaction mixture away from light. Prepare fresh reaction mixture for each experiment.

3. Alkaline phosphatase standard.

3.1 Prepare alkaline phosphate dilution buffer: Dilute 10X lysis buffer (Component D) to 1X with deionized water.

3.2 Dilute alkaline phosphatase standard (10 µg/mL - Component G) to 0.04 µg/mL (1:250) in dilution buffer. Then make five-fold serial dilutions to get the concentration of 0.008, 0.0016, 0.00032, 0.000064, 0.0000128, 0.00000256 and 0 µg/mL of alkaline phosphatase solution.

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

4. Detect alkaline phosphatase activity.

4.1 Add 50 µL (96-well plate) or 20 µL (384-well plate) of biological samples containing alkaline phosphatase to the wells.

4.2 Set up alkaline phosphatase standard: Add 50 µL (96-well plate) or 20 µL (384-well plate) serially diluted alkaline phosphatase standard solution from 0.04 to 0 ng/µL to the wells.

4.3 Set up the following controls:
Negative control: add same volume of biological samples, which do not contain alkaline phosphatase to selected wells.
Substrate control: add same volume of 1X lysis buffer to selected wells.

4.4 Add 50 µL/well (96-well plate) or 20 µL/well (384-well plate) of FDP reaction mixture to all the sample, standard and control wells on the plate. Mix the reagents by gently shaking the plate for 30 sec.

4.5 Measure fluorescence signal:
For kinetic reading: Immediately start measuring fluorescence intensity at Ex/Em=485 ±20 nm/528 ± 20 nm and continuously record data every 5 min for 15 to 30 min.
For end-point reading: Incubate reaction at the desired temperature for 15 to 30 min, and keep away from light. Optional: Add 50 µL/well (96-well plate) or 20 µL/well (384-well plate) of stop solution (Component C). Measure fluorescence intensity at Ex/Em=485 ±20 nm/528 ± 20 nm.

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

Protocol B. Detecting alkaline phosphatase activity in ELISA

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

1. **Prepare stock solution (for first time preparation only).**
   1.1 FDP stock solution: Reconstitute the substrate by adding 250 µL of DMSO (Component F) into the FDP vial (Component A). Mix the reagents thoroughly. Store the stock solution at -20°C, keep away from light.
2. **Prepare FDP reaction mixture.**
   2.1 Dilute 2X assay buffer (Component B) to 1X in deionized water.
   2.2 Dilute FDP stock solution (Component A) 1:200 with 2X assay buffer (Component B). Keep the reaction mixture away from light. Prepare fresh reaction mixture for each experiment.
3. **Detect alkaline phosphatase activity.**
   3.1 Set up the following controls:
   Substrate control: add 100 µL/well (96-well plate) or 50 µL/well (384-well plate) of 1X assay buffer to blank wells.
   3.2 Add 100 µL/well (96-well plate) or 50 µL/well (384-well plate) of FDP reaction mixture to the plate. Mix the reagents by gently shaking the plate for 30 sec.
   3.3 Measure fluorescence signal: Incubate reaction at the desired temperature for 30 to 60 min, and keep away from light. Optional: Add 50 µL/well (96-well plate) or 20 µL/well (384-well plate) of stop solution (Component C). Measure fluorescence intensity at Ex/Em=485 ± 20 nm/528 ± 20 nm.
   3.4 Data analysis: The fluorescence reading from the substrate control well is the background fluorescence. This background reading should be subtracted from the readings of the other wells. The fluorescence readings are expressed in relative fluorescence units (RFU).

Appendix I

Prepare cell extract for alkaline phosphatase.

- Prepare 1X lysis buffer by adding 1 mL of 10X lysis buffer (Component D) to 9 mL of deionized- water.
- Gently wash cells twice with 1X lysis buffer
- Add 20 µL of Triton X-100 (Component E) to 10 mL of 1X lysis 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 2500 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 E) and 1 mL of 10X lysis buffer (Component D) to 9 mL of deionized water.
- Homogenize tissue in 1X lysis buffer, and then centrifuge for 15 min at 10000x 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 $NaHCO_3$ in1 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 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 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 black high-binding ELISA plates for better signal to noise ratio.

3. ELISA:

1. Coating: add 100 µL of peptide-conjugate (PP-BSA) to each well of the 96-well plate at a concentration of 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 300 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.
3. Blocking: add 200 µL of blocking buffer and incubate 1h at room temperature.
4. Washing: repeat Step 2.
5. Add the primary antibody: dilute anti-peptide antibody in EIA buffer to appropriate concentration. Add 100µL of the diluted antibody to each well and incubate at room temperature for 1h on a plate shaker.
6. Washing: repeat Step 2.
7. Add the secondary antibody: dilute alkaline phosphatase conjugated secondary antibody in EIA buffer to the appropriate concentration (1:500 to 1:5000 dilution). Add 100 µL of diluted secondary antibody to each well and incubate at room temperature for 1h on a plate shaker.
8. Washing: repeat Step 2.
9. Detection by substrate: The plate is now ready for the FDP detection (refer to Protocol).

References

1. Kotobuki, N et al. *Cell Transplant.* **13**, 377 (2004).
2. Wyckoff M.H. et al. *J. Clin. Endocrinol. Metab* (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).