SensoLyte® FDP Alkaline Phosphatase ELISA Assay Kit *Fluorimetric*

Revision Number:1.1	*Last Updated: October 2014*
Catalog #	**AS-71101-R**
Kit Size	500 Assays (96-well plate)

- ***Convenient Format:*** Complete kit includes 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.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	FDP, fluorogenic 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	DMSO	0.3 mL
Component F	Alkaline phosphatase-conjugated goat anti-rabbit IgG	50 µL

Other Materials Required (but not provided)

- Microplate: Black, high-binding 96-well plate for ELISA.
- Fluorescence microplate reader: Capable of detecting emission at 520 nm with excitation at 420 nm.

Storage and Handling

- Except for Component A, store all components at 4°C.
- Store Component A at –20°C.

Introduction

The SensoLyte® FDP Alkaline Phosphatase Assay Kit is used in ELISA with alkaline phosphatase (AP) conjugated antibody or streptavidin. FDP (3,6-fluorescein diphosphate) is a sensitive fluorogenic substrate for alkaline phosphatase. The final hydrolytic product of FDP is fluorescein, a fluorophore that has a very high emission quantum yield. This characteristics of fluorescein makes FDP a superior substrate for alkaline phosphatase and increases the assay sensitivity by 100 times compared to the colorimetric substrate, *p*NPP[1]. The signal can easily be read by a fluorescence plate reader at Ex/Em=485±20/528±20 nm.

Protocol

Note 1: Prepare the ELISA assay plate according to standard ELISA procedures (refer to **Appendix**). Alkaline phosphatase-conjugated goat anti-rabbit IgG (Component E) is provided in this kit.

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

1. **Prepare FDP stock solution.**
   - FDP stock solution (200X): Reconstitute the substrate by adding 250 µL of DMSO (Component E) into the FDP vial (Component A). The stock solution is good for 3-4 months if stored at -20°C.

2. **Prepare the FDP reaction mixture.**
   - Prepare the FDP reaction mixture according to the following table. Keep the reaction mixture away from light.

Table 1. FDP reaction mixture for one 96-well plate (100 assays).

Components	Volume
FDP stock solution (200X)	50µL
Assay Buffer (Component B)	10 mL
Total volume	10mL

3. **Optional: if phosphate-buffered saline was used in ELISA procedures, the microplate must be washed with 1X wash buffer.**
   - Add 10 mL of 10 X wash buffer (Component D) to 90 mL deionized water to get 1X wash buffer.
   - Wash microplate with 200 µL/well of 1X wash buffer three times, then pad dry on paper towels. For better sensitivity, we recommend using the buffers described in Appendix.

4. **Start the alkaline phosphatase reaction.**
   - Add 100µL/well of FDP reaction mixture prepared in Step 2.
   - Incubate the reaction for 10 to 30 minutes, 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.

- Read the plate using a fluorescence microplate reader with a filter set of excitation/emission at 485±20/528±20 nm.
- The results can be plotted as shown in Figure 1.

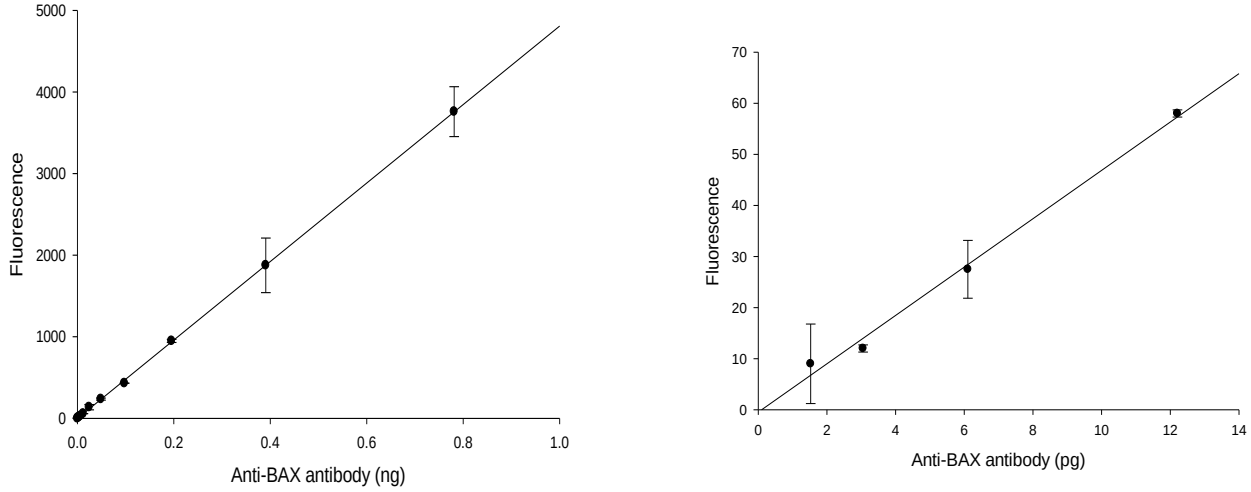

Figure 1. SensoLyte® FDP alkaline phosphatase ELISA assay kit was used to detect rabbit anti-BAX antibody.

The assay can detect as low as 1 pg (see right panel). The wells were coated with BAX-BSA. After blocking and washing, serially diluted rabbit anti-BAX antibody was then added into the wells. The wells were washed, and AP-conjugated goat anti-rabbit IgG secondary antibody (1:2000 dilution) was added. After incubation with secondary antibody the wells were washed and FDP reaction mixture was added. In 6 minutes the fluorescence was read by a fluorescence microplate reader at Ex/Em=485±20 nm/528±20 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 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. ELISA procedures:

1. Coating: Add 100 µL/well of protein to the 96-well plate at a concentration of 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). 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 30 min to 1 h on a plate shaker.
7. Washing: Repeat Step 3.
8. Detect by adding substrate: The plate is now ready for the FDP detection (refer to Protocol).

References

1. Huang, Z., Olson, N.A., You, W., Haugland, R.P. *J.Immunol.Methods* 149, 261-266 (1992).