SensoLyte® *p*NPP Secreted Alkaline Phosphatase Reporter Gene Assay Kit **Colorimetric**

Revision Number: 1.1	*Last updated: October 2014*

Catalog #	**AS-72144**
Kit Size	500 Assays (96-well plate)

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

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	*p*NPP, colorimetric placental alkaline phosphatase substrate	25 mL
Component B	10X Assay buffer	50 mL
Component C	Stop solution	25 mL
Component D	Triton-X-100	500 µL
Component E	Human Placental Alkaline Phosphatase Standard	10 µg/mL, 100 µL

Other Materials Required (but not provided)

- 96-well microplate: Clear microplates provide better signal to noise ratio
- Absorbance plate reader: Capable of detecting absorbance at 405 nm.

Storage and Handling

- Store component B and E at –20°C.
- Store other kit components at 4°C.
- Keep Component A away from light.

Introduction

Placental alkaline phosphatase is the most stable isoenzyme among the four mammalian alkaline phosphatase isoenzymes and it only exists naturally in the placenta of higher primates. These characteristics make placental alkaline phosphatase the enzyme of choice to serve as a reporter gene for the analysis of promoter activity and gene expression in cell culture or animals. The natural form of placental alkaline phosphates is membrane-anchored. The recombinant form of placental alkaline phosphatase, secreted alkaline phosphatase (SEAP)[1,2] can be efficiently secreted into tissue culture medium and serum. This unique characteristic of SEAP provides the advantage for performing kinetic analysis of gene expression over a period of time.

The SensoLyte® *p*NPP Secreted Alkaline Phosphatase Reporter Gene Assay Kit provides a convenient colorimetric assay of placental alkaline phosphatase for both secreted and membrane-bound forms by using *p*NPP as a phosphatase substrate. The absorbance signal can easily be read at 405 nm.

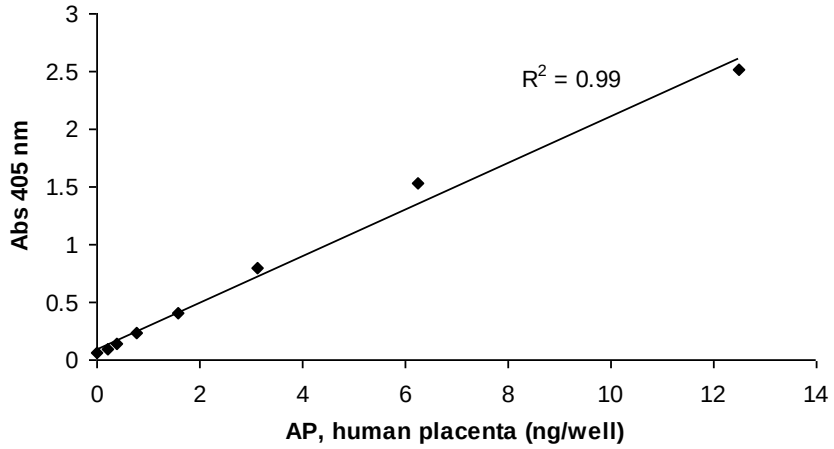

Figure 1. Detection of SEAP activity with *p*NPP. The detection limit can reach 0.1 ng (0.1 mUnit).

Protocol

Note: Bring all kit components to room temperature before starting the experiment.

1. Prepare working solutions.

1.1 Prepare placental alkaline phosphatase containing sample:

- Collect supernatants from transfected or control cells or prepare cell extracts if membrane-bound placental alkaline phosphatase was used for transfection (refer to Appendix for the preparation of cell extract).

Note: The supernatant or cell extract can be stored at -70°C for later use.

- Heat the culture supernatant or cell extract at 65°C for 10-30 min to inactivate endogenous non-specific alkaline phosphatase. Then cool down to room temperature.

1.2 *p*NPP placental alkaline phosphatase substrate (Component A): Ready to use.

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

1.4 Alkaline phosphatase standard: Dilute alkaline phosphatase standard (10 µg/mL - Component E) to 0.25 µg/mL (1:40) in dilution buffer. Then make two-fold serial dilutions to get the following alkaline phosphatase solution concentration: 125, 62.5, 31, 15.5, 7.75, 3.87, and 0 ng/mL .

2. Detect alkaline phosphatase activity.

2.1 Add 50 µL/well of supernatant or cell extract. Include a mock-transfected supernatant or cell extract to serve as a negative control.

2.2 Set up alkaline phosphatase standard (optional): Add 50 µL of serially diluted alkaline phosphatase standard solution from 250 to 0 ng/mL to the wells. The final amounts of alkaline phosphatase standard are 12.5, 6.25, 3.1, 1.55, 0.775, 0.387, 0.19 and 0 nanogram/well.

2.3 Add 50 µL of *p*NPP substrate solution into each well. Mix the reagents by gently shaking the plate for 30 sec.

2.4 Measure absorbance:

- For kinetic reading: Immediately start measuring absorbance at 405 nm and continuously record data every 5 min for 30 to 60 min.
- For end-point reading: Incubate reaction at the desired temperature for 30-60 min. Optional: Add 50 µL of Stop Solution (Component C) into each well. Shake the plate on a plate shaker for 1 min before the reading. Measure absorbance at 405 nm.

Note: If the amount of SEAP is low in the sample, the incubation time can be prolonged to overnight.

Appendix

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.

- Add 20 µL of Triton X-100 (Component D) to 10 mL of 1X assay buffer, mix well. Add an appropriate amount of 1X assay buffer with 0.2% Triton X-100 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 placental alkaline phosphatase assay.

References

1. Berger, J. et al. *Gene* 66, 1-10 (1988).
2. Cullen, BR. and MH. Malim, *Methods Enzymol.* 216, 362-368 (1992).