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

Catalog #	71233
Kit Size	500 Assays (96-well)

- ***Convenient Format:*** Complete kit including all the assay components.
- ***Optimized Performance:*** Optimal conditions for the detection of 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	*p*NPP, placental 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	0.5 mL

Other Materials Required (but not provided)

- 96-well microplate: A clear plate
- Absorbance microplate reader: Capable of detecting absorbance at 405 nm.

Storage and Handling

- Store Component A at –20°C.
- Store other kit components at 4°C.

Introduction

The placental alkaline phosphatase is the most stable isoenzyme among the four mammalian alkaline phosphatases 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 phosphatase is membrane-anchored. The recombinant form of placental alkaline phosphatase is the secreted alkaline phosphatase (SEAP).[1,2]

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 be read at 405 nm.

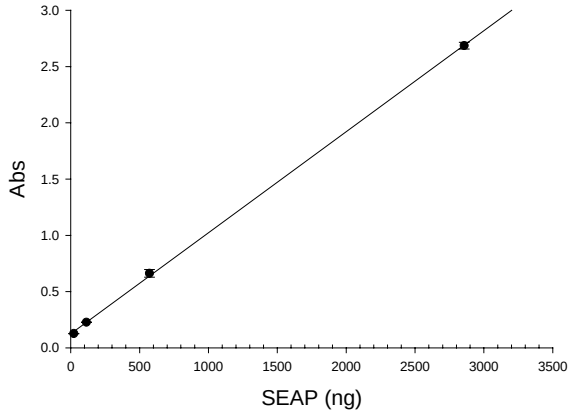

Figure 1. Detection of SEAP activity with *p*NPP. The detection limit can reach 10 ng (0.1 mUnit).

Protocol

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

1. Prepare placental alkaline phosphatase containing sample.

1.1 Collect the supernatant of tissue culture medium 48-72 hr after transfection of SEAP. Or prepare cell extract 48-72 hr after the transfection of membrane-bound placental alkaline phosphatase (refer to Appendix I for the preparation of cell extract).

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

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

2. Prepare stock solution (first time preparation only).

2.1 *p*NPP stock solution: Reconstitute by adding 250 µL of deionized water into the *p*NPP vial (Component A). Mix the reagents well. The stock solution will be good for 3-4 weeks if stored at –20°C.

3. Prepare *p*NPP reaction mixture.

3.1 Dilute *p*NPP stock solution 1:100 with 2X assay buffer (Component B). Prepare fresh reaction mixture for each experiment.

4. Start the placental alkaline phosphatase detection.

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

4.2 Add 50 µL/well of *p*NPP reaction mixture. Mix the reagents by gently shaking the plate for 30 sec.

4.3 Measure absorbance:

- For kinetic reading: Immediately start measuring absorbance at 405 nm continuously and record data every 5 min for 30-60 min.
- For end-point reading: Incubate the reaction for 30-60 min. Optional: Add 50 µL/well (96-well plate) or 20 µL/well (384-well plate) of stop solution (Component C). 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 incubate time can be prolonged to overnight.

Appendix I

Prepare cell extract for membrane-bound placental alkaline phosphatase

- Dilute 10X lysis buffer (Component D) to 1X in deionized water.
- Wash cells with 1X lysis buffer twice.
- 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 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 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. J. Berger, J. Hauber, R. Hauber, R. Geiger, B. R. Cullen, *Gene* 66, 1-10 (1988).
2. B. R. Cullen and M. H. Malim, *Methods Enzymol.* 216, 362-368 (1992).