SensoLyte® Luminescent Secreted Alkaline Phosphatase Reporter Gene Assay Kit *Luminometric*

Revision Number: 1.1	*Last updated: October 2014*

Catalog #	**AS-72145**
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 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	Human Placental Alkaline Phosphatase Standard	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 component B and C at –20°C.
- Store other kit components at 4°C.
- Protect Component A from light.

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® Luminescent Secreted Alkaline Phosphatase Reporter Gene Assay Kit provides a convenient method to detect placental alkaline phosphatase activity for both secreted and membrane-bound forms by using a chemiluminescent substrate. The assay can detect femtogram level of alkaline phosphatase. The kit provides ample materials to perform 500 assays in a 96-well plate format. The protocol can readily be modified to run assays in a 384-well format.

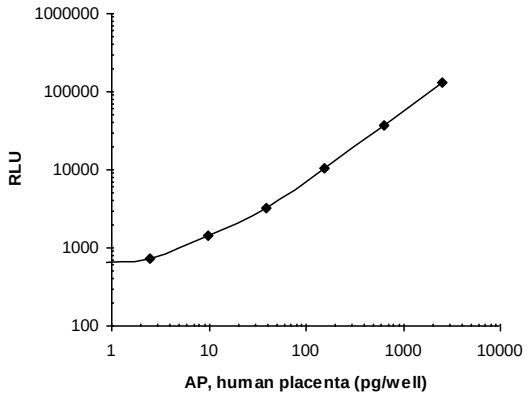

Figure 1. Calibration curve for human placental alkaline phosphatase with the chemiluminescent substrate.

Protocol

Note: Warm 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 Chemiluminescent alkaline phosphatase substrate (Component A): Ready to use.

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

1.4 Prepare dilutions of alkaline phosphatase: Dilute alkaline phosphatase standard (10 µg/mL - Component C) to 0.05 µg/mL (1:200) in dilution buffer. Then make four-fold serial dilutions to get the concentration of 12.5, 3.125, 0.78, 0.195, 0.05, and 0.012 ng/mL of alkaline phosphatase solution. Include a blank control.

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

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 50 to 0 ng/mL to the wells. The final amounts of alkaline phosphatase standard are 2500, 625, 156, 39, 9.8, 2.44, 0.6, and 0 picogram/well.

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

2.4 Measure luminescent signal:

- For kinetic reading: Immediately start measuring luminescent 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.

2.5 Data analysis: The luminescence reading from the blank 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).

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.
- 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 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 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 10000x g at 4°C. Collect the supernatant for alkaline phosphatase assay.

Reference

1. Berger, J. et al. *Gene* 66, 1 (1988).
2. Cullen, BR. et al. *Methods Enzymol.* 216, 362 (1992).