aCella™ - SEAP

Secreted Alkaline Phosphatase (SEAP)
Chemiluminescent Reporter Gene Assay Kit

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Notes
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CELL TECHNOLOGY

I. Introduction:

Secreted alkaline phosphatase (SEAP) is widely used as a reporter to study gene expression. SEAP is secreted into the culture supernatant of the transfected cell lines. SEAP activity in the culture medium reflects changes in intracellular concentrations of SEAP mRNA and protein. The changes of gene expression can be easily assayed using the same cultures. Cell Technology's aCella^{TM} - SEAP detection kit utilizes a Chemiluminescent-based assay technique to detect SEAP reporter gene expression. It provides an extremely sensitive tool to quantitate SEAP and analyze transcriptional and promoter activity in transfected cells.

In the assay, a chemiluminescent substrate was used to detect the Secreted Alkaline Phosphatase in cell culture or serum. This kit is extremely sensitive and ideal for use in quantifying alkaline phosphatase in samples with low volume, or samples that contain interfering agents with chromogenic assays. In addition, the elimination of cell lysis permits repeated sampling of the cell medium for analysis.

II. Assay Principle:

The reaction utilizes a stabilized 1, 2-dioxetane substrate, which provide high signal to noise ratio, wide dynamic range, rapid results and excellent reproducibility. The SEAP enzyme transfers the phosphoryl residue via a phosphoryl-enzyme intermediate. The intermediate is not stable and decomposed and emits light for detection. The assay can detect alkaline phosphatase at attogram level. The kit provides sample material for 100 assays in a 96-well plate format.

III. Storage:

Refrigerated at 2-8°C, away from light. Long term: Aliquoted samples can be stored for 2 years at 2-8°C (do not freeze).

IV. Warnings and Precautions:

1. For research use only. Not for use in diagnostic procedures.
2. Practice safe laboratory procedures by wearing protective clothing and eyewear.

V. SEAP Kit Part # 5063: Kit Contents (for 100 assays):

Kit Part # 5063:

1. **Part # 6030:** Alkaline phosphatase**:** One vial of 100X stock, 100 µl.
2. **Part # 3062:** 10 X SEAP Assay buffer, one vial of 2 ml.
3. **Part # 7024:** Chemiluminescent substrate.

Materials required but not supplied:

1. 96-well cell culture plate
2. Luminescent plate
3. Luminescent plate reader
4. Deionized water

VI. Preparation of Reagent Working Solutions:

1. **Prepare 10 ml of 1X** SEAP Assay **buffer:** 1 ml of 10 X SEAP Assay Buffer (Part # 3062) is added to 9 ml of deionized water to make 1X SEAP Assay buffer. This should be sufficient for performing 100 assays.

2. **Alkaline phosphatase (AP) (Part # 6030):** 100 X. Prepare for a working solution of AP enzyme by adding 50 µl AP stock to 4.5 ml of 1 X SEAP Assay Buffer. This should be sufficient for performing 100 assays.

3. Store at 4°C. Do not freeze!

4. Chemiluminescent substrate **(Part # 7024):** It is ready to use. Store at 2-8^{0}C. Equilibrate at room temperature for 30 min before use. Do not contaminate the substrate with any phosphate buffer.

VII. Assay Protocol:

1. To prepare alkaline phosphatase (AP) standard curve, 10 µl 100 X standard stock at 10 U/ml (Part# 6030**)** is supplied with the kit. Dilute the appropriate amount of 100X AP stock in 1X SEAP assay buffer to make standard curve concentrations ranging from 0 to 50 mU/ml in well.

   Label suitable tubes 1-8. To tube #1, add 990 µL of 1X SEAP assay buffer (Part# 3062) and 10µL of the AP standard (Part# 6030**)**. This will make a 100 mU/ml solution of AP. Next serially dilute (1:2) the 100 mU/ml AP standards in 1X SEAP assay buffer to construct a standard curve. This can be accomplished by adding 500µL of 1X SEAP assay buffer into tubes #2- 8. From tube #1, remove 500µL of the 100 mU/ml AP standard and add it to tube #2 containing 500µL 1X SEAP assay buffer to give 50 mU/ml AP. Gently vortex tube #2 and pipette out 500µL from tube #2 and add it to tube#3 containing 1 x SEAP assay buffer. Continue this process to tube #7. Tube# 8 is the blank control. The final AP concentration in the well will be 2 times less than in the tube.

Tube #	AP concentration in tubes	Final AP concentration in wells
1	100 mU/ml	50 mU/ml
2	50 mU/ml	25 mU/ml
3	25 mU/ml	12.5 mU/ml
4	12.5 mU/ml	6.25 mU/ml
5	6.25 mU/ml	3.125 mU/ml
6	3.125 mU/ml	1.56 mU/ml
7	1.56 mU/ml	0.78 mU/ml
8	0	0

2. Be sure to include a positive and negative control in the assay if a standard curve is not used.

3. Cell culture preparation:

Transfect cells with the construct that expresses SEAP. Control cells should be included as a separate sample.
Culture the cells in a CO_2 incubator for 24 hours or for the period of time that you used for your experimental protocol. Sample supernatant will be used for the assay. We recommend testing a few dilutions of the sample (with 1X SEAP buffer) to ensure that some readings fall within the range of the standard curve.

4. Pipette 50µL of the AP standard dilutions, controls and samples into the wells of a 96-well plate.

5. Add 50µL of the Chemiluminescent Substrate (Part# 7024) to each well to begin the reaction.

6. Measure luminescent signal:

   For kinetic reading: Immediately start measuring luminescent intensity and record data continuously every 5 min for 15-30 min.
   For end-point reading: Incubate the reading for 15 to 30 min. Keep plate away from direct light.

7. Data analysis:

   Subtract background luminescence from each reading.
   Make a plot of Relative Luminescence Units (RLU) as a function of AP activity and determine the equation of the line.

   Use AP standard curve to calculate the AP level in the sample. If the reading is out of the detection range of the standard curve, dilution of the sample with 1X SEAP assay buffer may be needed.

VIII. Sample Data:

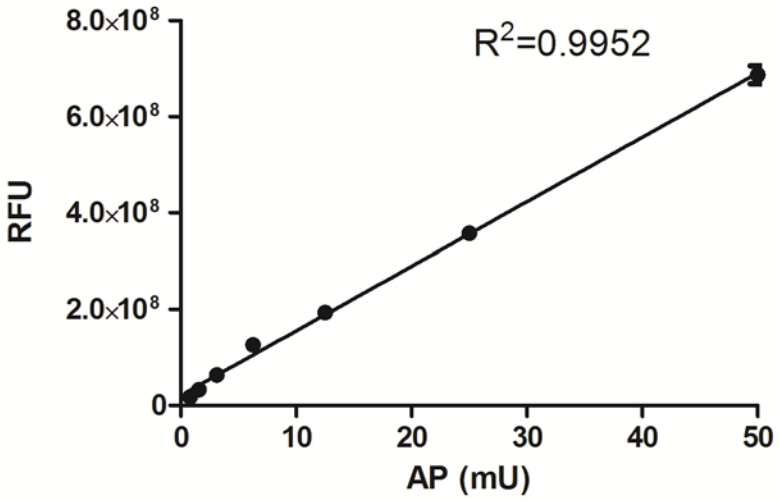

Figure 1. Standard curve of Alkaline Phosphatase (AP). 50 µl serial dilutions of AP (Starting dose 100 mU in tubes) were added to the wells of 96-well luminescent plate. 50 µl of Chemiluminescent substrate was added and the plate was read immediately with a luminometer.

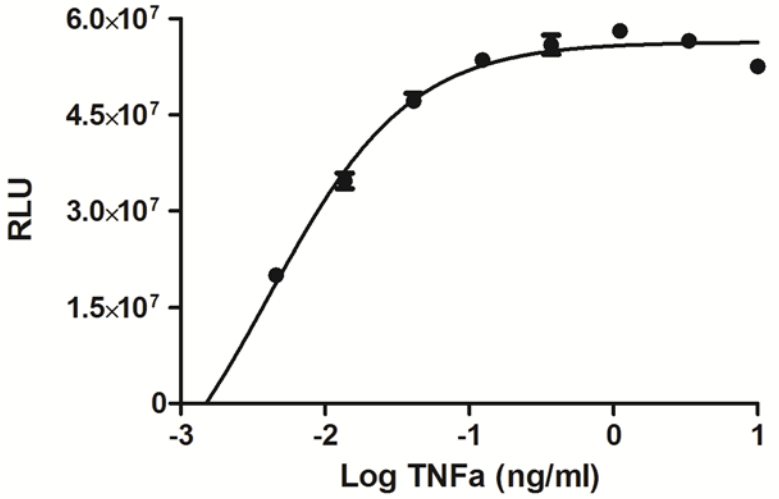

Figure 2. Detection of the functional activity of NF-κB/SEAP stable reporter cell line. NF-κB/SEAP reporter cells (IML-101) were plated in 96-well plates at 50,000 cells/well. After 24 hours of incubation, cells were stimulated with different doses of TNFα for another 24 hours. 50 µl culture supernatant was added to the wells of 96-well white opaque luminescent plate. 50 µl of chemiluminescent substrate was added and the plate was read immediately with a luminometer.

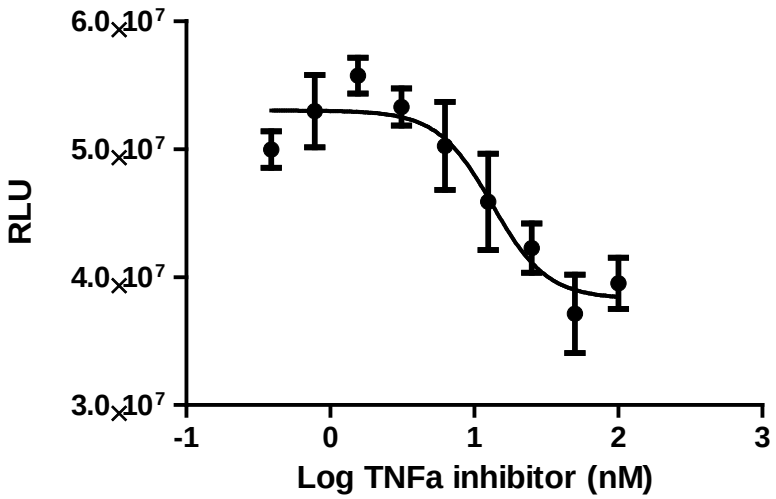

Figure 3. Inhibition of TNFα activity by a quinazoline TNFα inhibitor (EMD Millipore Cat# 545380-34-5) with NF-κB/SEAP stable reporter cell line. NF-κB/SEAP reporter cells (IML-101) were plated in 96-well plates at 50,000 cells/well. After 24 hours, 10 ng/ml of TNFα and different dose of TNFα inhibitors were added into each wells. Cells were incubated at 37°C for 24 hours. 50 µl culture supernatant was added to the wells of 96-well white opaque luminescent plate. Then 50 µl of Chemiluminescent substrate was added and the plate was read immediately with a luminometer.

References:

1. Hiramatsu N, Kasai A, Hayakawa K, Nagai K, Kubota T, Yao J, Kitamura M. (2006). Secreted protein-based reporter systems for monitoring inflammatory events: critical interference by endoplasmic reticulum stress. *J Immunol Methods* 315 (1-2):202-207.

2. Kain, S.R. (1997). Use of secreted alkaline phosphatase as a reporter of gene expression in mammalian cells. *Methods Mol Biol* 63: 49-60.

3. Andre CM, JM Greenwood, Walker EG, Rassam M, Sullivan M, Evers D, Perry NB, Laing WA. (2012). Anti-inflammatory procyanidins and triterpenes in 109 apple varieties. *J Argric Food Chem* 60:10546-10554.