Promega

Application Note

A GloMax® 96 Microplate Luminometer Method for Alkaline Phosphatase

1. INTRODUCTION

The GloMax® 96 Microplate Luminometer has a large dynamic range that is highly suited for alkaline phosphatase assays. Alkaline phosphatase is a stable enzyme often conjugated to secondary antibodies for immunoassays. Alkaline phosphatase is also a useful genetic reporter and a helpful tool in DNA cloning.

The GloMax® 96 can detect as little as 3×10^{-21} moles alkaline phosphatase. Measurements are linear over four orders of magnitude (Figure 1). All tests were conducted using Lumigen's APS-5 (Lumigen, Southfield, MI) and purified calf intestinal mucosa alkaline phosphatase (Biozyme, San Diego, CA).

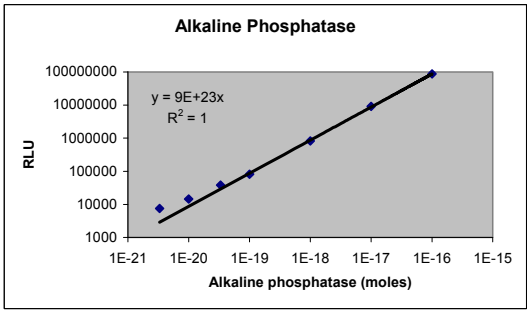

Figure 1: Detection of alkaline phosphatase with the GloMax® 96 Microplate Luminometer. 90 µL APS-5 was added to 10 µL alkaline phosphatase diluted in dH_2O. Samples were incubated at room temperature for 5 minutes before measurement.

2. MATERIALS REQUIRED

- GloMax® 96 Microplate Luminometer
- 96-well plates, white (E&K Scientific EK-25075)
- Lumigen APS-5
- Alkaline phosphatase (Biozyme ALPI12G)
- p200 pipette and pipette tips
- p20 pipette and pipette tips

3. PROTOCOL

3.1 Reagent Preparation

Lumigen APS-5: Use as supplied. Store at 2–8°C, where it is stable for up to 12 months.

Alkaline phosphatase: Store at 4°C.

3.2 Instrument Setup

3.2.1 Double-click on the GloMax® 96 icon to start the software.

3.2.2 Click on "Create New Protocol" from the "Welcome to GloMax® 96" dialog box.

3.2.3 Using the new protocol wizard, set up a protocol with 0 injectors, 1 second integration and 5 minute delay. Select the wells you wish to read and click on "Finish".

3.2.4 Enter your information into the "Experiment", "Operator", "Plate No.", and "Notes" fields in the "Main Dialog Box".

3.3 Sample Analysis

3.3.1 Equilibrate alkaline phosphatase, samples and APS-5 to room temperature.

3.3.2 Prepare a 10-fold serial dilution of alkaline phosphatase in your assay buffer.

3.3.3 Add 10 µL of sample or standard to the 96-well plate.

3.3.4 Add 90 µL of APS-5 reagent to the sample or standard.

Note: The recommended ratio of sample volume to APS-5 volume is 1:10 and should not exceed 1:1

3.3.5 Insert the plate into the GloMax® 96 and click on "Start" to begin assay. After the programmed 5-minute delay, the GloMax® 96 will begin measurement. RLU values measured by the GloMax® 96 will appear in the Excel spreadsheet after all the selected wells in each row have been read. If you encounter an error message, refer to the troubleshooting guide in the GloMax® 96 Technical Manual for more information.

Note: It is not recommended to open another Excel spreadsheet while the GloMax® 96 reads your sample plate.

3.3.6 Once the measurements are complete, you can access Excel to analyze your data.

3.3.7 Be sure to remove your plate after measurement.

CONTACT INFORMATION

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