Promega

Application Note

A GloMax® 96 Microplate Luminometer Method for the Beta-Glo® Assay

1. INTRODUCTION

The GloMax® 96 Microplate Luminometer in combination with the Beta-Glo® Assay System provides a reliable, homogeneous method to quantitate ß-galactosidase expression in mammalian cells. The Beta-Glo® Assay generates a bright, glow-type signal that remains stable for more than 4 hours. The prolonged luminescence allows for batch processing of multiple plates. The Beta-Glo® Assay System couples the ß-galactosidase cleavage of 6-O-ß-galactopyranosyl-luciferin with the firefly luciferase reaction to generate light[1]. The amount of light produced is proportional to the amount of ß-galactosidase present (Figure 1).

The extended dynamic range of the GloMax® 96 Microplate Luminometer allows the user to easily measure very dim and very bright samples on the same plate using the Beta-Glo® Assay System. A pre-installed Beta-Glo® template on the GloMax® 96 facilitates quick set-up. The GloMax® 96 Microplate Luminometer detects as little as 30 fg ß-galactosidase using Beta-Glo® Reagent. Measurements are linear from 30 fg to 1 ng ß-galactosidase or more than 4 orders of magnitude (Figure 1).

The Beta-Glo® Reagent is compatible with commonly used culture media for mammalian cells (RPMI 1640, MEMα, DMEM, and Ham's F12) containing 0-10% serum. The luminescent signal is affected by the presence of phenol red, temperature changes and organic solvents. Results should be compared only between samples measured with similar media/serum mixtures. For optimal performance, minimize the presence of phenol red and organic solvents (i.e., DMSO), which will decrease luminescence.

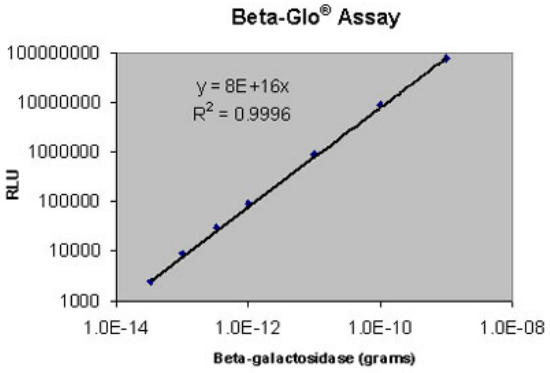

Figure 1. Beta-Glo® Assay performed on the GloMax ® 96 Microplate Luminometer. ß-galactosidase was diluted in 25 mM HEPES buffer containing 0.1% gelatin. Following the addition of Beta-Glo® Reagent, the microplate was incubated at room temperature for 60 minutes before measurement.

2. MATERIALS REQUIRED

- GloMax® 96 Microplate Luminometer
- 96-well plates, white (E&K Scientific EK-25075)
- Beta-Glo® Assay System (Cat.# E4720, E4740, E4780)
- p200 pipette and pipette tips

3. PROTOCOL

3.1 Reagent Preparation

Beta-Glo® Assay Substrate: Use as supplied. Store at -20°C.

Beta-Glo® Assay Buffer: Use as supplied. Store at -20°C.

Beta-Glo® Assay Reagent: Transfer the contents of one bottle of Beta-Glo® Assay Buffer to one bottle of Beta-Glo® Assay Substrate. Mix by inversion until the substrate is thoroughly dissolved. Use reconstituted reagent on the same day it is prepared to generate best

performance. Reconstituted reagent may be stored at 22°C for up to 2 days with ≤ 20% loss of potency and 4°C or -20°C for up to 7 days with ≤ 10% loss of potency. Store reagent away from light.

Note: The temperature of the Beta-Glo® Assay Reagent should be held constant at room temperature while quantifying luminescence since luciferase activity is temperature dependent. Reagent stored frozen after reconstitution must be thawed below 25°C to ensure performance. Mix well after thawing. The simplest method for thawing is to place the reagent in a water bath at room temperature.

3.2 Instrument Setup

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

3.2.2 Click on "Run Promega Protocol" from the "Welcome to GloMax® 96" dialog box.

3.2.3 Select "BetaGlo" from the list of Promega protocols.

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

3.2.5 Click "Options" from the "Main Dialog Box" to select the wells to be read and modify the number of runs. Once modified, click on "Apply Changes" and return to the "Main Dialog Box".

3.3 Sample Analysis

3.3.1 Remove the 96-well plate containing cell cultures from the incubator.

Note: For maximum reproducibility, equilibrate cell cultures to room temperature before adding reagent.

3.3.2 Add a volume of Beta-Glo® Assay Reagent equal to that of the culture medium in each well. For 96-well plates, typically 100 µL of reagent is added to cells grown in 100 µL of medium. For optimal results, do not reduce the volume of reagent to less than a 1:1 ratio with the volume of medium.

3.3.3 Use a plate shaker to mix the sample contents for 30 seconds. Thorough mixing is necessary for maximum reproducibility.

3.3.4 Allow the sample to incubate at room temperature for at least 30 minutes.

Note: The initial ramp-up period for the luminescent signal to reach maximum light intensity is 30—60 minutes. Between 30—60 minutes, the rate of increase in luminescence is ≤ 20% per 10-minute period. The change in luminescent signal between 60—240 minutes is ≤10% per 60-minute period.

3.3.5 Insert the plate into the GloMax® 96 Microplate Luminometer and click on "Start" to begin assay. RLU values measured by the GloMax® 96 Microplate Luminometer 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 for more information.

Note: Opening another Excel spreadsheet while the GloMax® 96 reads your sample plate may cause loss of data and is not recommended.

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

Note: Please remove your plate after measurement.

CAUTION: The lyophilized Beta-Glo® Substrate contains dithiothreitol (DTT) and is therefore classified as hazardous. The reconstituted reagent is not known to

present any hazards as the concentration of DTT is less than 1%. However, we recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents. Promega assumes no liability for damage resulting from handling or contact with these products.

4. REFERENCES

1. Geiger, R. *et al*. (1992) A new ultra sensitive bioluminogenic enzyme substrate for ß-galactosidase. *Biol. Chem. Hoppe-Seyler* **373**, 1187-91.

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