

A GloRunner™ Microplate Luminometer Method for Promega Beta-Glo®



1. INTRODUCTION

The Turner BioSystems GloRunner™ Microplate Luminometer in combination with Promega's Beta-Glo® Assay System provides a reliable, homogeneous method to quantitate β -galactosidase expression in mammalian cells. Promega's Beta-Glo® Assay System 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 β -galactosidase cleavage of 6-O- β -galactopyranosyl-luciferin with the firefly luciferase reaction to generate light¹. The amount of light detected by the GloRunner™ Microplate Luminometer is proportional to the amount of β -galactosidase present (Figure 1).

The GloRunner™ Microplate Luminometer detects as little as 100 fg β -galactosidase using Beta-Glo® Reagent. Measurements are linear from 100 fg to 1 ng β -galactosidase or four orders of magnitude (Figure 1).

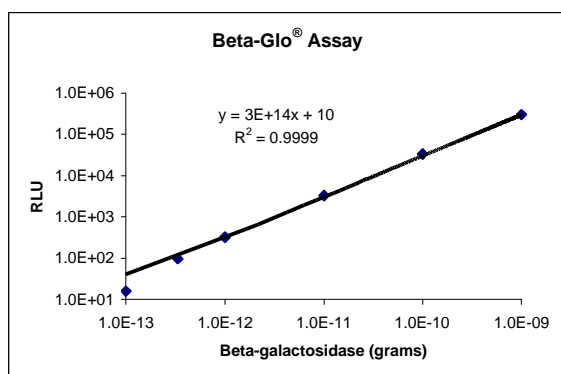


Figure 1. Beta-Glo® Assay performed on the GloRunner™ Microplate Luminometer. Beta-galactosidase was diluted in 25 mM HEPES buffer containing 0.1% gelatin. Following the addition of Beta-Glo® Reagent, the microplate was incubated at room temperature for 60 minutes before measurement.

The Beta-Glo® Reagent is compatible with commonly used culture media for mammalian cells (RPMI 1640, MEM α , 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 otherwise decrease luminescence.

2. MATERIALS REQUIRED

- GloRunner™ Microplate Luminometer (P/N 9000-000)
- 96-well plates, white (E&K Scientific EK-25075)
- Beta-Glo® Assay System (Promega Corporation E4720, E4740, or E4780)
- p200 pipette and pipette tips

3. EXPERIMENT 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 \leq 20% loss of potency and 4°C or -20°C for up to 7 days with \leq 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 reagent performance. Mix well after thawing. The simplest method for thawing is placing the reagent in a water bath at room temperature.

3.2 Instrument Setup

3.2.1 Double click on the GloRunner™ icon to start the software.

3.2.2 Open a new workbook and select the wells you wish to read from the “Options” screen.

3.2.3 You may also wish to select a delay for Incubation, the number of repeated runs, and a delay before microplate ejection. The recommended measurement duration is 1 second.

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 the Beta-Glo[®] Assay Reagent equal to that of the culture medium in each well. For 96-well plates, typically 100 μ L of reagent is added to cells grown in 100 μ L 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 $\leq 20\%$ per 10-minute period. The change in luminescent signal between 60—240 minutes is $\leq 10\%$ per 60-minute period.

3.3.5 Insert the plate into the GloRunner™ Microplate Luminometer and click "Start" to begin assay. The data will appear in the Excel spreadsheet after the GloRunner™ completes the run.

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

Note: Please remove your plate after measurement.

4. REFERENCES

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

5. ABOUT PROMEGA CORPORATION

Beta-Glo is a trademark of Promega Corporation and is registered with the U.S. Patent and Trademark Office. Orders for Promega's products may be placed by:

Phone: (800) 356-9526
Fax: (800) 356-1970
E-mail: custserv@promega.com

Mailing Address:

Promega Corporation
2800 Woods Hollow Rd.
Madison, WI 53711 USA

6. ABOUT TURNER BIOSYSTEMS

GloRunner is a trademark of Turner BioSystems. Orders for Turner BioSystems' products may be placed by:

Phone: (408) 636-2400 or
Toll Free: (888) 636-2401 (US and
Canada)
Fax: (408) 737-7919

Web Site: www.turnerbiosystems.com

E-Mail: sales@turnerbiosystems.com

Mailing Address:

Turner BioSystems, Inc.
645 N. Mary Avenue
Sunnyvale, CA 94085 USA

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 and Turner BioSystems assume no liability for damage resulting from handling or contact with these products.