Glo and Go with Beta-Glo™ Reagent

Introducing the Beta-Glo™ Assay System

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Abstract

The Beta-Glo™ Assay System is a luminescent homogeneous reagent for quantitation of β-galactosidase in cells. The Beta-Glo™ Reagent generates a bright luminescent signal and is several-fold more sensitive than other currently available assays. The single-step "add, mix and measure" format of the assay is designed for convenient analysis of multiwell plates, yet it can also be adapted to single-tube formats. The luminescent signal remains stable for several hours after reagent addition and covers a wide dynamic range extending four logs. These features make the Beta-Glo™ Assay a flexible and powerful tool for studying a wide range of cellular events.

The Beta-Glo™ Assay System is a simple, sensitive and flexible assay for quantitation of β-galactosidase in both single-tube and multiwell formats.

Introduction

β-galactosidase is widely used as a reporter molecule and as a co-reporter with firefly luciferase. It is used in protein-protein interaction studies based on yeast two-hybrid systems and in enzyme complementation assays. Numerous substrates for quantitation of β-galactosidase are commercially available; these substrates are typically based upon luminescent, fluorescent or colorimetric formats. We describe here Promega's new Beta-Glo™ Assay System, a homogeneous reagent that provides a bright luminescent signal that is stable over several hours without prior sample processing.

Overview of the Beta-Glo™ Assay

The Beta-Glo™ Assay System[a,b] (Cat.# E4720, E4740 and E4780) uses a coupled enzyme reaction in which the substrate (6-O-β-galactopyranosyl luciferin) is cleaved by β-galactosidase to yield free luciferin that is used in a reaction catalyzed by luciferase to generate a luminescent signal proportional to the amount of β-galactosidase present (Figure 1). Since a single reagent lyses cells and contains all of the components required to generate the luminescent signal, high-throughput processing of samples is possible.

Brightness and Linear Range

One of the most noticeable features of the Beta-Glo™ Reagent is the high level of luminescence generated. Figure 2A demonstrates that the Beta-Glo™ Reagent generates a 3-fold brighter luminescence than other commercially available homogeneous assays for β-galactosidase. The Beta-Glo™ Reagent signal also has a 4-log linear range extending from 100fg to 1ng of enzyme concentration in a 96-well plate (Figure 2B). Thus, the Beta-Glo™ Reagent is several hundred-fold more sensitive than standard colorimetric assays based on O-nitrophenyl-β-D-galactopyranoside (ONPG). The bright luminescent signal generated by Beta-Glo™ Reagent, coupled with exceptional sensitivity, can facilitate reporter quantitation using CCD imagers, low-volume formats (e.g., 384-well plates) and in samples with low levels of expression.

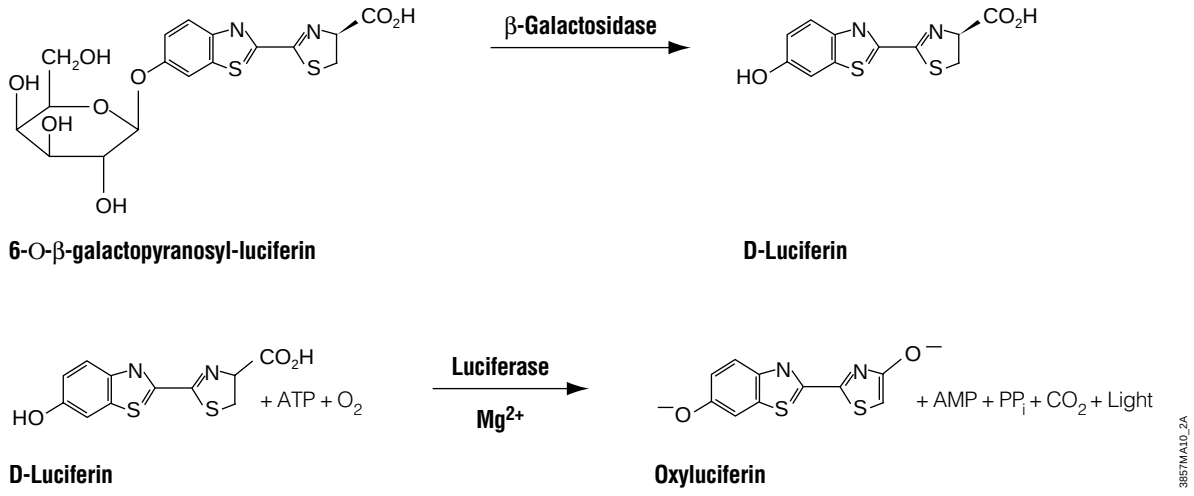

Figure 1. Coupled enzyme reaction of the Beta-Glo™ Assay System.

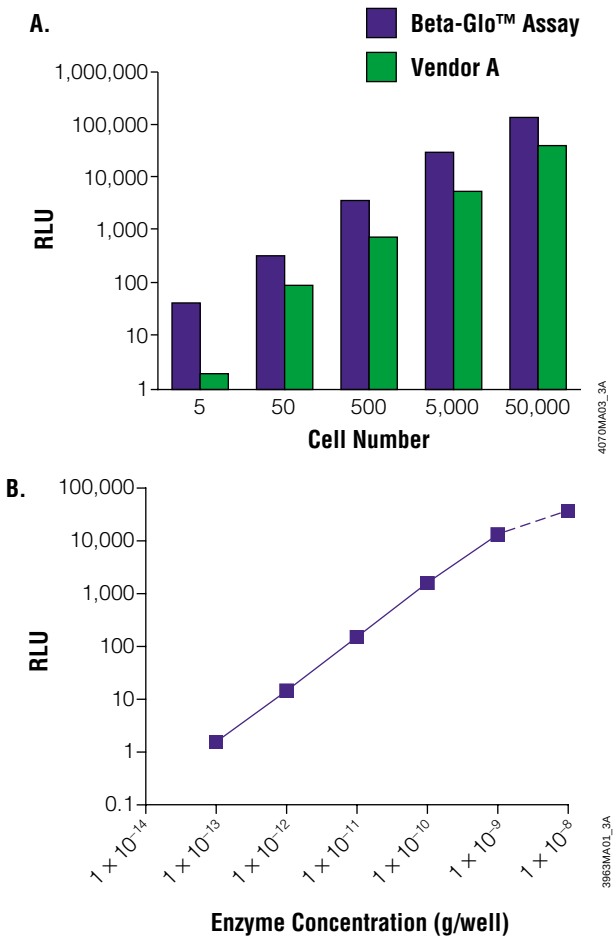

Figure 2. Beta-Glo™ Assay brightness and linear range. Panel A. Varying numbers of stably expressing ψ2BAG cells were assayed for β-galactosidase activity using either the Beta-Glo™ Assay System or Vendor A's luminescent assay system per the manufacturer's instructions. Results were measured using a Berthold Orion luminometer at 1 second per well. **Panel B.** Purified β-galactosidase was serially diluted in 25mM HEPES and 0.1% Prionex®. One hundred microliters per well was added to a 96-well plate. An equal volume of Beta-Glo™ Reagent was added, and luminescence was measured 30 minutes later using a Dynex MLX® luminometer at 0.5sec/well. All readings were corrected for background luminescence. n = 3 for all data points.

Signal Kinetics

The Beta-Glo™ Reagent generates a stable luminescent signal that may be measured 30 minutes after reagent addition and as long as 4 hours. This stability allows flexibility in processing samples. The luminescent signal has an initial ramp-up period in which maximum light intensity is generally reached between 30 and 60 minutes after reagent addition (Figure 3). In this interval, the maximum rate of change of luminescent signal is generally <20% per 10-minute period. For measurements using 96-well plate formats at a 1-second read per well, the maximum rate of change within the plate would generally be less than 5%. The luminescent signal after 60 minutes shows a change per hour of ≤10% for up to 4 hours. This signal stability facilitates batch or continuous processing of multiwell plates using any luminometer.

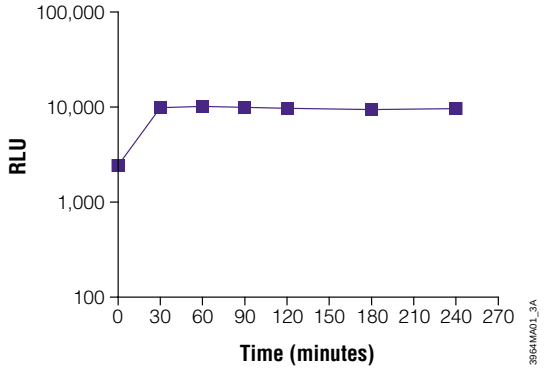

Figure 3. Beta-Glo™ Assay signal kinetics. Purified β-galactosidase (1×10^{-9}g [0.5mU]; Sigma Cat.# G5635) was added to 25mM HEPES and 0.1% Prionex® in a 96-well plate for a total volume of 100µl per well. The Beta-Glo™ Reagent was added to the well contents and the signal was measured over a 4-hour period using a Dynex MLX® luminometer at 0.5 seconds/well. Results are the average of 3 replicates and are corrected for background.

Reagent Robustness

The Beta-Glo™ Assay uses a volume of Beta-Glo™ Reagent that is equal to the volume of culture medium in each well/tube. Thus, the added reagent constitutes half of the chemical environment in which the assay is performed. Organic solvents such as DMSO and ethanol are often used to solubilize chemical compounds prior to their testing in cell-based assays. In addition, typical luminescent reactions depend on the rate of enzyme catalysis and can vary with changes in temperature. Both reaction temperature and organic solvents could be expected to have an adverse impact on assay performance. However, we have seen that the Beta-Glo™ Reagent is exceptionally robust with respect to these conditions.

We tested the effects of temperature changes on luminescence by performing parallel Beta-Glo™ reactions at room temperature (22°C) and at 27°C. The results of the background-corrected values indicate that the average luminescent signal generated by the Beta-Glo™ Reagent differs by less than 11% at both temperatures (data not shown). We also evaluated the Beta-Glo™ Reagent for compatibility with a range of solvents. Solvent concentrations ranging from 0.5% to 2% (prior to reagent addition) had little impact on Beta-Glo™ Reagent luminescence and resulted in a loss of signal of less than 10% (data not shown).

Performing the Beta-Glo™Assay

The Beta-Glo™ Assay System consists of two components (a lyophilized substrate and an assay buffer) that are combined to form the Beta-Glo™ Reagent (Figure 4). The Beta-Glo™Assay is optimal when performed at room temperature (~22°C), eliminating the need for incubations at elevated temperature, as required with other assay formats. A volume of Beta-Glo™ Reagent is added to an equal volume of cells in medium, mixed and then incubated at room temperature for 30–60 minutes for the luminescent signal to reach peak activity. The Beta-Glo™ Reagent has been shown to be compatible with a wide range of mammalian cell types in addition to bacterial lysates. However, for bacterial lysates, Reporter Lysis Buffer (Cat.# E3971) has been shown to yield optimal results. Once the signal has reached peak luminescence, it can be measured for up to 4 hours after reagent addition.

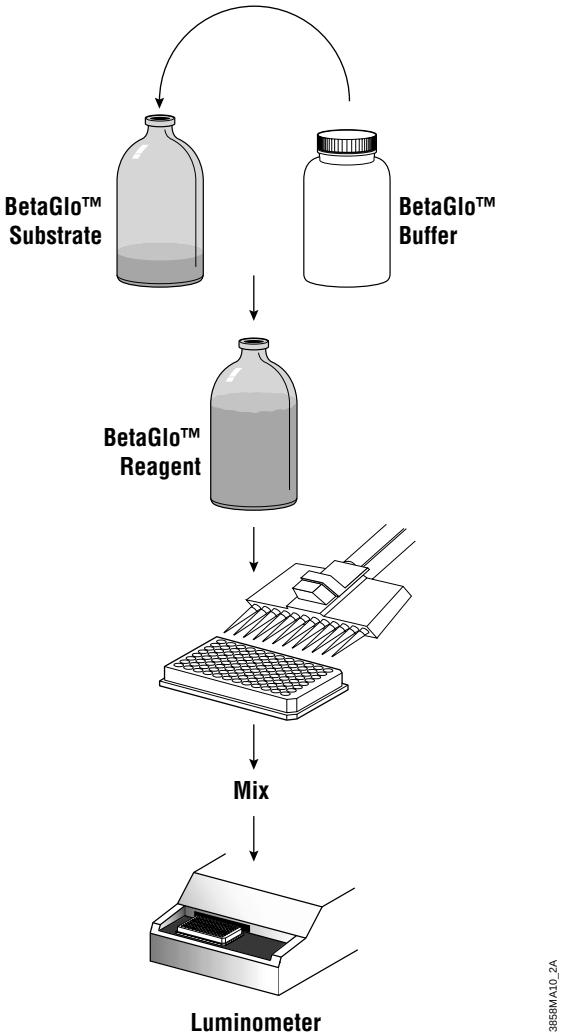

Figure 4. The Beta-Glo™ Assay protocol. The Beta-Glo™ Assay Substrate and Buffer are combined to make Beta-Glo™ Reagent. A volume of reagent equal to the volume of culture medium is added to cells/medium. Samples are incubated at room temperature for a minimum of 30 minutes and analyzed in a luminometer.

Summary

The Beta-Glo™ Assay System provides a simple, sensitive, flexible and robust assay for quantitation of β-galactosidase. The extended luminescence of the single-step, room temperature procedure provides flexibility for single-tube reactions as well as continuous or batch processing of multiwell plates.

Protocol

- *Beta-Glo™ Assay System Technical Manual* #TM239, Promega Corporation. (www.promega.com/tbs/tm239/tm239.html)

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Ordering Information

Product	Size	Cat.#
Beta-Glo™ Assay System[a,b]	10ml	E4720
	100ml	E4740
	10 × 100ml	E4780

[a] Certain applications of this product may require licenses from others.

[b] The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.