BETA-GLO® ASSAY SYSTEM: A LUMINESCENT β-GALACTOSIDASE ASSAY FOR MULTIPLE CELL TYPES AND MEDIA

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The Beta-Glo® Assay System provides a sensitive luminescent reagent for detecting and quantifying β-galactosidase activity in a homogeneous assay format. It is brighter and more sensitive than any other currently available luminescence-based β-galactosidase assay. After reaching peak activity, the reaction exhibits steady-state kinetics for several hours, making it suitable for high-throughput screening. The assay is linear over a wide range of cell number, spanning at least four orders of magnitude of enzyme activity. This homogeneous format is amenable to any multiwell plate format and can be used with mammalian, yeast or bacterial cells.

Chemistry and Assay Procedure

The Beta-Glo® Assay can be used to measure β-galactosidase activity in a variety of biological applications, including complementation studies involving protein:protein interaction and yeast two-hybrid screening. The Beta-Glo® Assay System[a,b] is a homogeneous bioluminescent assay that couples β-galactosidase activity to a luciferase reaction (Figure 1). β-galactosidase catalyzes a reaction in which the substrate (D-luciferin-*o*-β-galactopyranoside) is cleaved to release luciferin. This luciferin serves as a substrate for luciferase that is present in the reagent. As a result of the luciferase activity, oxyluciferin is formed, and light is emitted. The luminescence observed is proportional to the amount of β-galactosidase present. The reagent has been carefully formulated so that the assay is a single-step procedure that involves adding an equal volume of reagent to a sample that contains the enzyme either in solution or present in cells grown in medium and serum. For the latter purpose, the reagent also contains a detergent that lyses cells to release the β-galactosidase present.

Compatibility with Mammalian and Yeast Cells

The Beta-Glo® Assay was developed for use with mammalian cells. We used a cell line (ψ2BAGα) that was derived from NIH3T3 cells and stably transfected with bacterial β-galactosidase. We show that the luminescence produced is linear from 3 cells to 30,000 cells per well in a 96-well plate (Figure 2). The luminescence is stable, and there is virtually no loss in activity from 30 minutes to 4 hours after adding the reagent (2). The stable kinetics allows processing of multiple plates at a time.

Additionally, the reagent was tested for its ability to be used with yeast cells; it was shown to be an effective reagent for measuring β-galactosidase activity in the yeast two-hybrid system, even in a highly miniaturized format. Figure 3A shows that the reagent can lyse yeast cells and is also brighter than another commercially available reagent specifically designed for use with yeast cells; the Beta-Glo® Assay also appears to have greater sensitivity. This is particularly important for detection of weaker interactions where a less sensitive reagent might be unable to detect low signals (Figure 3B). The signal from a yeast two-hybrid assay was shown to be stable between the recommended time point after Beta-Glo® Reagent addition (25 minutes) to over one hour (Figure 4).

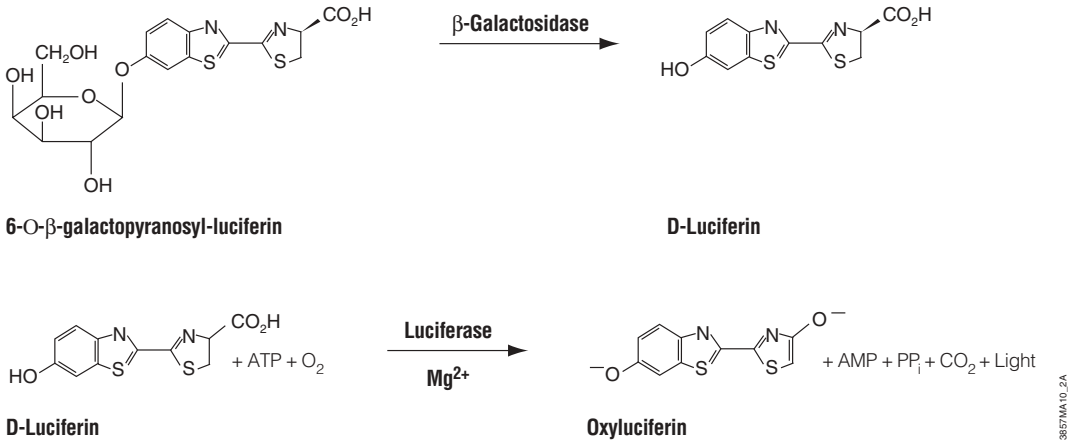

Figure 1. Summary of the coupled reactions in the Beta-Glo® Assay System. β-galactosidase activity from lysed cells or in solution catalyzes the conversion of D-luciferin-*o*-β-galactopyranoside to D-luciferin, which is in turn a substrate for luciferase.

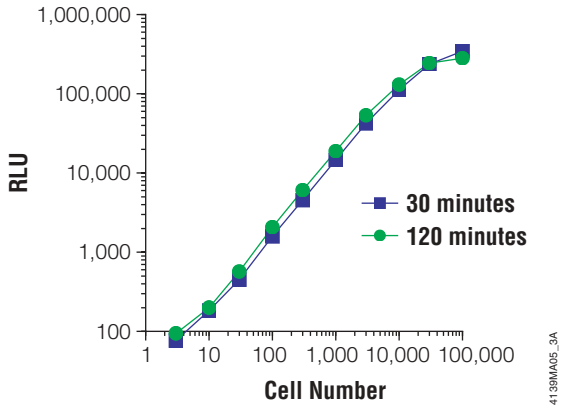

Figure 2. Linear range of the Beta-Glo® Assay System. Varying numbers of ψ2BAGα cells (described in the text) were plated into 96-well plates in 100µl of DMEM + 10% CS and allowed to attach. An equal volume (100µl) of Beta-Glo® Reagent was added, and the plates were agitated for 15–30 seconds to mix the contents of the wells. The plate was maintained at room temperature for 30 minutes, and luminescence was read in a Dynex MLX® luminometer at 30 and 120 minutes after reagent addition. Results represent the mean of eight replicates for each point.

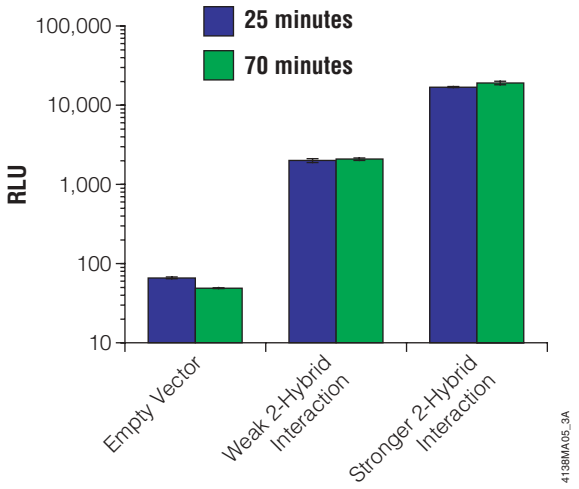

Figure 4. Stability of Beta-Glo® Assay in a 384-well format. Yeast cells (15µl) were incubated for 25 minutes with 15µl of the reagent, and luminescence was imaged for 30 seconds in a ViewLux™ Imager at 25 and 70 minutes after reagent addition. Results represent the mean of 4 replicates and standard errors.

The reagent has been carefully formulated so that the assay is a single-step procedure.

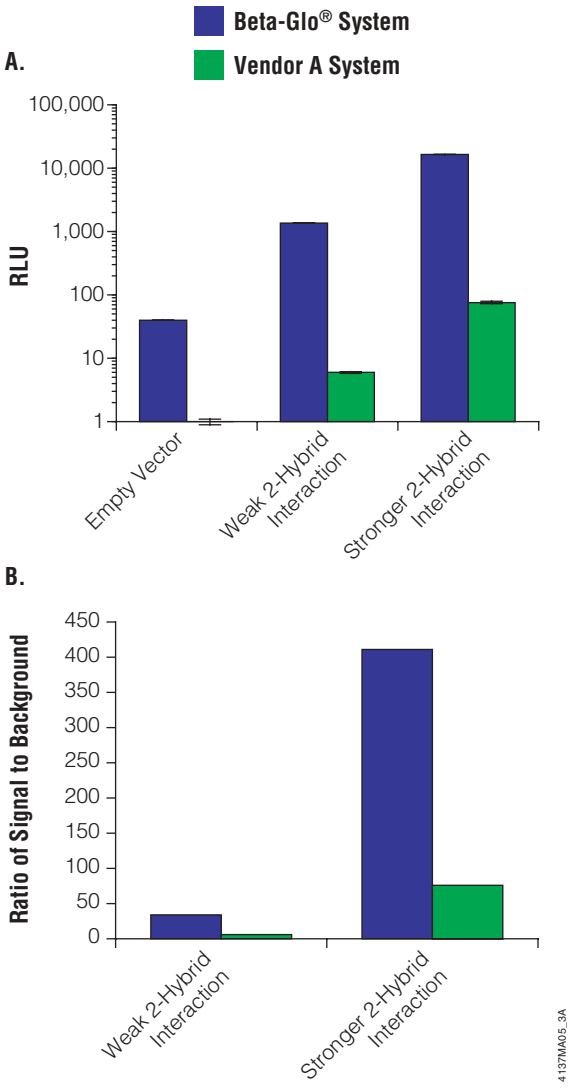

Figure 3. Beta-Glo® Assay in 1,536-well format. Panel A. Yeast (*S. cerevisiae*) two-hybrid strains in 1µl volume were incubated for 25 minutes with 1µl reagent, and luminescence emitted was measured in a ViewLux™ Imager (Perkin Elmer). Results represent a mean of at least 8 replicates with standard errors. **Panel B.** Signal-to-background ratio showing better detection of weak yeast two-hybrid interaction with Beta-Glo® Assay System than Vendor A, a comparable homogeneous luminescent β-galactosidase assay. Background is the signal obtained from the yeast containing the empty vector. Results represent a mean of at least eight replicates.

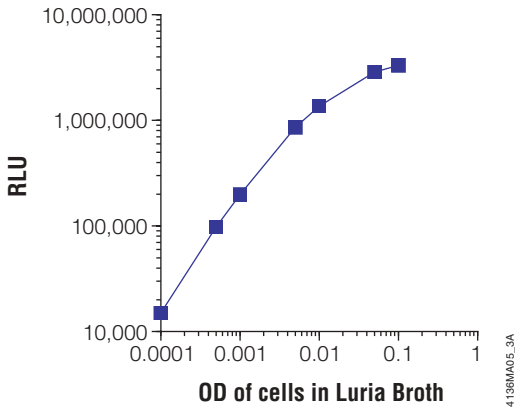

Figure 5. β-galactosidase activity in bacterial cells. JM109 cells containing pGEM®3Zf(+) Vector were diluted in LB to varying absorbance values at 600nm. One hundred microliters of the diluted cultures were dispensed into 96-well plates, and then an equal volume of reagent was added. The plates were agitated briefly to mix, and luminescence was read 30 minutes later. These results are the average of triplicates for each cell density. The replicates varied by less than 5% at each cell density.

Compatibility with Bacterial Cells and Lysates

Preliminary testing with JM109 bacterial cells containing the pGEM®3Zf(+) Vector[c] indicated that the Beta-Glo® Assay can lyse bacterial cells in Luria Broth (LB) growth medium. The assay for β-galactosidase activity was carried out at varying densities of bacterial cells. The emitted light was proportional to the number of cells present (based on OD_{600}; Figure 5). Neither the addition of lysozyme nor a freeze-thaw step increased the β-galactosidase signal with these bacterial cells (data not shown).

Additionally, the reagent was also tested for compatibility with various buffers. Figure 6 shows that the assay works well for β-galactosidase activity in buffers used for sample preparation or in media used for growing cells. The Beta-Glo® Assay was also tested against several lysis buffers (Promega reagents) that are used to prepare cell lysates for reporter assays. The most compatible buffers appear to be H/P

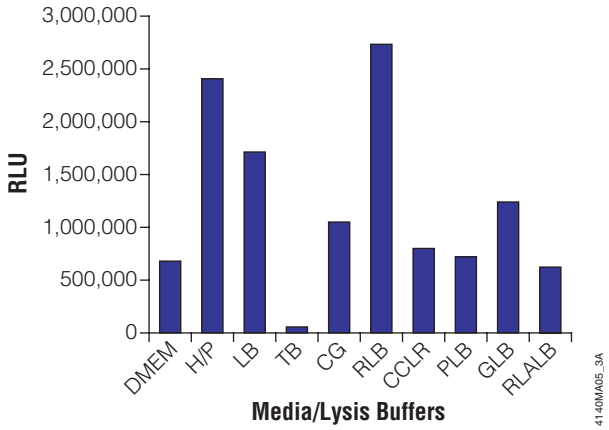

Figure 6. Compatibility of Beta-Glo® Reagent with various buffers and growth media. One-tenth milliunit of β-galactosidase in 100µl of each test buffer was dispensed into the wells of a 96-well plate. The assay was performed according to the protocol described in the the *Beta-Glo® Assay System Technical Manual* #TM239, and light output was read at 3.5 hours following reagent addition. Buffers tested: Dulbecco's Modified Eagle's Medium (DMEM); 25mM HEPES + 0.1% Prionex® (H/P); Luria Broth (LB); Terrific Broth (TB); CircleGrow® (CG), Reporter Lysis Buffer (RLB); Cell Culture Lysis Buffer (CCLR); Passive Lysis Buffer (PLB); Glo Lysis Buffer (GLB); and *Renilla* Luciferase Assay Lysis Buffer (RLALB). These results are the average of triplicates for each buffer or medium tested, and the replicates varied by less than 5% for each condition.

(HEPES with Prionex®), Luria Broth (LB) and Reporter Lysis Buffer (RLB).

Summary

The Beta-Glo® Assay is based upon a simple, flexible, and robust technology that can be applied to a variety of conditions. The assay can be carried out in multiwell plate formats as dense as 1,536-well plates. The extended, stable kinetics of the assay make it user-friendly for high-throughput users because the reagent may be used in batch or continuous processing mode without injectors. Although this assay was developed for mammalian cells, it also works well with yeast and bacterial cells and is compatible with other lysates containing β-galactosidase activity. ■

References

1. Geiger, R. *et al.* (1992) *Chem. Hoppe-Seyler* **373**, 1187–91.

2. Browning, J. *et al.* (2003) *Promega Notes* **84**, 16–18.

Protocol

Beta-Glo® Assay System Technical Manual #TM239 (www.promega.com/tbs/tm239/tm239.html)

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.

[c]U.S. Pat. No. 4,766,072.