Technical Manual

Beta-Glo® Assay System

INSTRUCTIONS FOR USE OF PRODUCTS E4720, E4740 AND E4780.

PRINTED IN USA.
Revised 9/11

www.promega.com

Part# TM239

Beta-Glo® Assay System

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1. Description

The Beta-Glo® Assay System[a,b,c] is a homogeneous method used to quantitate β-galactosidase expression in mammalian cells. High-throughput quantitation of β-galactosidase expression in mammalian cells is commonly performed by batch processing of 96- and 384-well plates. The Beta-Glo® Assay System has been developed specifically for this purpose, providing a bright luminescent signal that is stable over several hours in commonly used cell culture medium without prior sample processing. The homogeneous assay procedure (Figure 1) involves the addition of a single reagent directly to cells cultured in serum-supplemented medium. Throughput rates of several thousand samples per hour may be achieved with high reproducibility under standard laboratory conditions. The luminescent signal generated by the Beta-Glo® Reagent may be measured after 30 minutes for more than 4 hours in common culture medium (Figure 2). The extended luminescence eliminates the need for reagent injectors and provides flexibility for continuous or batch processing of multiple plates.

The Beta-Glo® Assay System consists of two components that are combined to form Beta-Glo® Reagent. This single reagent provides a coupled enzyme reaction system utilizing a luciferin-galactoside substrate (6-O-β-galactopyranosyl-luciferin). This substrate is cleaved by β-galactosidase to form luciferin and galactose (1). The luciferin is then utilized in a firefly luciferase reaction to generate light (Figure 3). Since a single reagent lyses cells and contains all of the components required to generate a luminescent signal that is proportional to the amount of β-galactosidase, many plates can be processed quickly and efficiently.

The Beta-Glo® Reagent is extremely sensitive and has a wide dynamic range. As shown in Figure 4, the Beta-Glo® Reagent is capable of detecting 100fg of β-galactosidase enzyme and has a linear range extending over 4 logs of enzyme concentration (10^{-13} to 10^{-9}g).

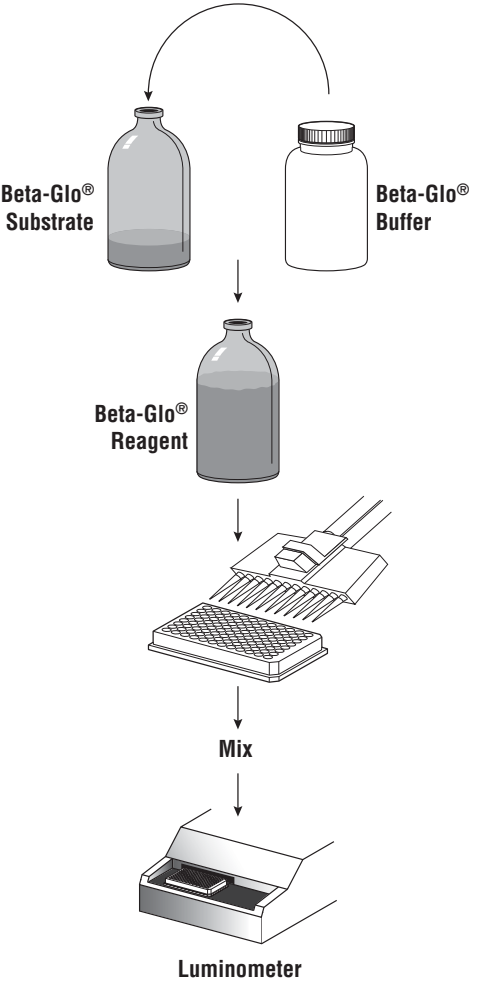

Figure 1. Beta-Glo® Assay System protocol. The Beta-Glo® Assay Substrate and Buffer are combined to make Beta-Glo® Reagent. A volume of reagent equal to the culture medium is added to cells in culture medium. Samples are incubated at room temperature for at least 30 minutes and analyzed in a luminometer.

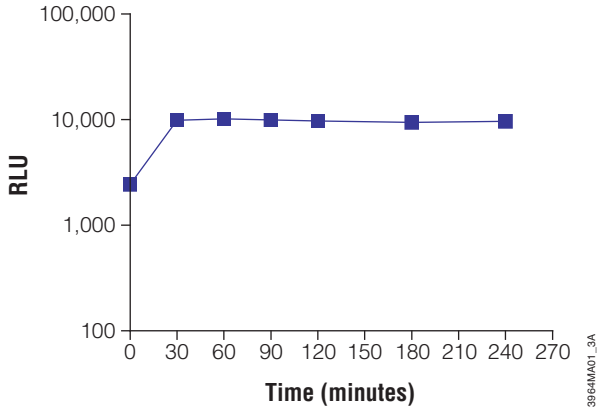

Figure 2. Beta-Glo® Assay signal kinetics. Purified β-galactosidase (1.0×10^{-9}g [0.5mU]; Sigma Cat.# G5635) was added to 25mM HEPES and 0.1% Prionex® (hydrolyzed porcine gelatin) in a 96-well plate at 100µl per well. Beta-Glo® Reagent was added to the well contents and the signal was measured over 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.

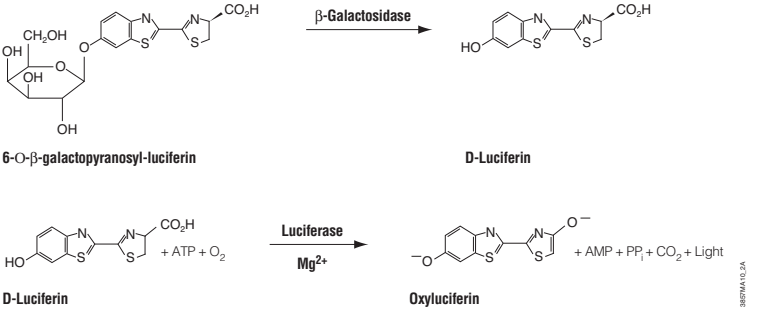

Figure 3. Coupled enzyme reaction of the Beta-Glo® Assay System. The amount of β-galactosidase present in a sample correlates with the amount of luminescence generated by that sample. In the reaction, 6-O-β-galactopyranosyl-luciferin is cleaved by β-galactosidase to yield luciferin, which is then catalyzed by luciferase in the presence of cofactors to yield light.

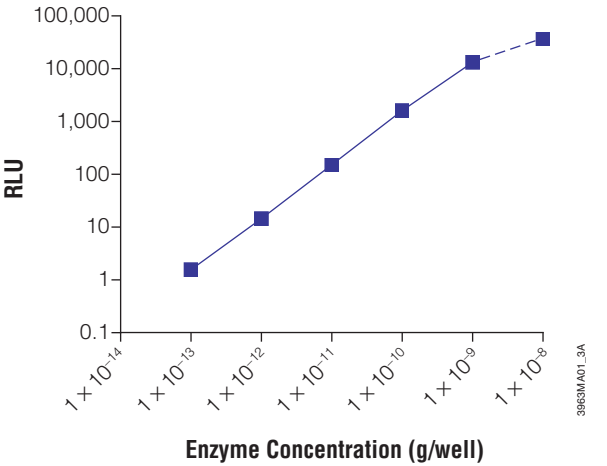

Figure 4. Sensitivity and range of the assay. Purified β-galactosidase serially diluted in 25mM HEPES and 0.1% Prionex® was added to 96-well plates at 100µl per well. An equal volume of Beta-Glo® Reagent was added and luminescence was measured 30 minutes later using a Dynex MLX® luminometer at 0.5 seconds/well. Results are the average of 3 replicates and are corrected for background.

2. Product Components and Storage Conditions

Product	Size	Cat#
Beta-Glo® Assay System	10ml	E4720

Each system contains sufficient reagent to perform 100 assays of 100µl each. Includes:

- 1 vial Beta-Glo® Assay Substrate
- 10ml Beta-Glo® Assay Buffer

Product	Size	Cat#
Beta-Glo® Assay System	100ml	E4740

Each system contains sufficient reagent to perform 1,000 assays of 100µl each. Includes:

- 1 vial Beta-Glo® Assay Substrate
- 100ml Beta-Glo® Assay Buffer

Product	Size	Cat#
Beta-Glo® Assay System	10 × 100ml	E4780

Each system contains sufficient reagent to perform 10,000 assays of 100µl each. Includes:

- 10 vials Beta-Glo® Assay Substrate
- 10 × 100ml Beta-Glo® Assay Buffer

Storage Conditions: Store the Beta-Glo® Assay Substrate and Assay Buffer at –20°C. The substrate should be stored away from light. See product label for expiration date information. Approximate stability of Beta-Glo® Assay Reagent after reconstitution: A loss of potency of ≤20% when stored at 22°C for up to 2 days; ≤10% loss of potency when stored at 4°C or –20°C for up to 7 days. The Reagent may be subjected to 3 freeze-thaw cycles with ≤10% change in potency. Best performance will be generated from freshly prepared Beta-Glo® Reagent. Store Reagent away from light.

3. Performing the Beta-Glo® Assay

3.A. General Considerations

The Beta-Glo® Assay System can be used with the following culture media containing 0–10% serum: RPMI 1640, MEMα, DMEM and Ham's F12. Other media/sera combinations may be used, but experimental verification of assay performance is recommended. The luminescent signal can also be affected by the presence of phenol red, organic solvents and changes in temperature.

The luminescent signal is affected by assay conditions; therefore, results should be compared only between samples measured using the same medium/serum combination. For analysis of multiwell plates, we recommend including a common control sample on each plate. Using this method, luminescence measurements of each plate can be normalized to the control contained within the sample plate as well as between plates. This allows for correction of small variations in luminescence that can occur over time or due to other variables such as temperature.

The Beta-Glo® Reagent should be added to plates at least 30 minutes before measuring luminescence. This time is required for the luminescent signal to stabilize.

Note: The Beta-Glo® Assay Reagent is not intended for use with automated reagent injectors that are integrated into some luminometers.

3.B. Reagent Preparation

To prepare the Beta-Glo® Assay Reagent, transfer the contents of one bottle of Beta-Glo® Assay Buffer to one bottle of Beta-Glo® Assay Substrate. Gently mix by inversion until the substrate is thoroughly dissolved.

Notes:

1. The temperature of the Beta-Glo® Assay reaction should be held constant while quantitating luminescence, since both enzyme reactions are temperature dependent. This is easily achieved by using Reagent equilibrated to room temperature. Temperature equilibration of the Reagent is unnecessary when the Buffer is stored at room temperature the night before Reagent preparation.
2. Reagent stored frozen after reconstitution should be thawed below 25°C to ensure optimal reagent performance. Mix well after thawing. The most convenient and effective method for thawing or equilibrating cold reagent is to place it in a water bath maintained at room temperature.

3.C. Assay Procedure

1. Remove 96- or 384-well plates containing mammalian cells from the incubator. The plates must be compatible with the luminometer being used.
2. For maximum reproducibility, equilibrate cultured cells to room temperature before adding Beta-Glo® Reagent.
3. Add a volume of reagent equal to the volume of culture medium in each well. For 96-well plates, typically 100µl of reagent is added to cells growing in 100µl of culture medium. For 384-well plates, usually 25µl of reagent is added to 25µl medium.
4. Mix the sample contents for 30 seconds using a plate shaker. Thorough sample mixing is required for maximum assay reproducibility. Alternatively, some automated pipettors may provide sufficient mixing upon reagent delivery, but this should be verified for individual circumstances.
5. Incubate the samples for at least 30 minutes at room temperature to allow for the signal to stabilize, then measure using a luminometer (consult instrument manual). Generally, an integration time of 0.25–1 second per well is sufficient.

Notes:

1. We recommend using white-walled or white-bottom tissue culture-compatible plates for luminescence measurements.
2. To achieve linear assay performance at low β-galactosidase expression levels, background luminescence must be subtracted from all readings. Background luminescence may be caused by endogenous galactosidases present in serum and mammalian cells. Wells containing medium and serum or nontransfected cells in medium and serum provide background measurements for use in background subtraction. Some luminometers also require verification of linear response at high light levels.
3. The luminescent signal has an initial ramp-up period in which maximum light intensity is generally reached between 30 and 60 minutes of reagent addition. Between 30 and 60 minutes after reagent addition, the maximum rate of change of the luminescent signal is generally ≤20% per 10-minute period. For measurements using 96-well plates at a 1-second read per well (approximately 2.5 minutes to read the entire plate), the maximum rate of change of luminescence within the plate would be generally less than 5%. However, using a 384-well plate at a 1-second read per well (approximately 7 minutes to read an entire plate), the maximum change of luminescence across the plate would be generally less than 10%. We recommend reading samples in a consistent time frame if you measure between 30 and 60 minutes and using controls on each plate to normalize these and other effects. The luminescent signal after 60 minutes generally shows a ≤10% change in luminescent signal per hour for up to 4 hours after reagent addition.

4. When using purified β-galactosidase enzyme as a positive control or to generate a standard curve, be aware that different sources of enzyme can give rise to different signal kinetics.

3.D. Conditions Affecting Assay Performance

Temperature

All Beta-Glo® Assays should be performed at room temperature. The intensity and rate of decay of the luminescent signal is dependent on the rates of the coupled enzyme reaction, which can vary at different temperatures. To obtain consistent results, perform the assay at room temperature.

Reagent Mixing

For best results, mix the contents of the wells containing sample and reagent using a plate shaker.

Assay Volume

For best results, do not reduce the volume of reagent to less than a 1:1 ratio with the volume of medium.

Chemical Environment

Different combinations of media and sera can give rise to different luminescent characteristics (e.g., light intensity, signal kinetics and background). The presence of 0.5% phenol red in the cell culture medium may also have an impact on the background-corrected luminescent signal, resulting in an approximate twofold decrease in luminescence. Additionally, organic solvents can have an adverse impact on the assay. For example, a 1% solution of DMSO in the samples will generally reduce the background-corrected luminescent signal by less than 10%. For best results, minimize the presence of phenol red and organic solvents in samples.

4. Appendix

4.A. Overview of the Beta-Glo® Assay System

Transcriptional regulation, coupled to the expression of a reporter gene, is routinely used to study a wide range of physiological events. One example is the analysis of receptor function by quantifying the action of specific response elements on gene expression. Other examples include the study of signal transduction pathways, transcription factors, protein:protein interactions and viral infection and propagation (2,3). Events downstream of transcription, such as mRNA processing and protein folding, can also be analyzed (4–8).

β-galactosidase has been used as a reporter gene for many years. It is a tetrameric protein with a molecular subunit weight of approximately 116kDa. β-galactosidase catalyzes the hydrolytic cleavage of galactose from glucose in a lactose molecule. In the Beta-Glo® Assay System, the ability of β-galactosidase to catalyze the hydrolytic cleavage of galactose from luciferin in 6-O-β-galactopyranosyl-luciferin is exploited (1). Firefly luciferase in the Reagent uses the liberated luciferin and other cofactors present (magnesium and ATP) to form oxyluciferin with the concomitant emission of light.

Use of Lysis Buffers

If there is a need for making a lysate before using the Beta-Glo® Assay System, we have demonstrated that it is compatible with Reporter Lysis Buffer (Cat.# E3971; see reference 9)

4.B. Related Products

Product	Size	Cat.#
pSV-β-Galactosidase Control Vector	20µg	E1081
X-Gal*	100mg	V3941
PureYield™ Plasmid Midiprep System	25 preps	A2492
	100 preps	A2495
β-Galactosidase Enzyme Assay System with Reporter Lysis Buffer	65 assays	E2000
Bright-Glo™ Luciferase Assay System	10ml	E2610
	100ml	E2620
	10 × 100ml	E2650
Steady-Glo® Luciferase Assay System	10ml	E2510
	100ml	E2520
	10 × 100ml	E2550
TransFast™ Transfection Reagent	1.2mg	E2431
Transfectam® Reagent for the Transfection of Eukaryotic Cells	1mg	E1231
	0.5mg	E1232
Tfx™-20	4.8mg	E2391
Tfx™-50	2.1mg	E1811
ProFection® Mammalian Transfection System—Calcium Phosphate	40 reactions	E1200

* For Laboratory Use.

4.C. References

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[a]U.S. Pat. No. 6,602,677 and Australian Pat. No. 754312 have been issued to Promega Corporation for thermostable luciferases and methods of production. Other patents are pending.

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

[c]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.

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MLX is a registered trademark of Dynex Technologies, Inc. Prionex is a registered trademark of Pentapharm Ltd.

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Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

Promega Corporation · 2800 Woods Hollow Road · Madison, WI 53711-5399 USA
Toll Free in USA 800-356-9526 · Phone 608-274-4330 · Fax 608-277-2516 · **www.promega.com**
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