Add, Mix and Measure Microbial Growth

BacTiter-Glo™ Assay for Antimicrobial Drug Discovery and General Microbiology

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Abstract

The BacTiter-Glo™ Microbial Cell Viability Assay determines the number of viable cells in culture by measuring ATP. This simple assay can provide results in as little as five minutes, and its excellent sensitivity allows you to detect microbial growth sooner than with conventional O.D. measurements. It is easily adaptable to automated and multiwell formats, and automated reagent injectors are not required.

The superior features of the BacTiter-Glo™ Assay support its use in a variety of applications from routine examination of microbial growth to antimicrobial drug discovery.

Introduction

The BacTiter-Glo™ Microbial Cell Viability Assay[a,b] is a homogeneous ATP-based bioluminescent assay for detecting and quantitating microbial cells. The assay uses a single reagent to release and measure ATP from microbial cells. The characteristics of this assay are described in detail in *Promega Notes* 88 (1) and in the *BacTiter-Glo™ Microbial Cell Viability Assay Technical Bulletin*, #TB337. In brief, the assay protocol follows a simple "add-mix-measure" format (Figure 1). The assay generates stable luminescent signals, is effective on a variety of microbes, detects as few as ten bacterial cells and has a typical dynamic range of over five orders of magnitude. It is also compatible with typical microbial culture media and organic solvents.

These superior features provide the basis for developing a variety of applications using the BacTiter-Glo™ Assay, from routine examination of microbial growth to antimicrobial drug discovery. The add-mix-measure format and the stable luminescent signal make the BacTiter-Glo™ Assay an ideal choice for high-throughput screening. Therefore, it is particularly useful in the discovery and development of new antibiotics to combat infectious diseases caused by microbial pathogens and to address the spread of antibiotic resistance. In this article, we demonstrate some of these applications.

Examine Bacterial Growth with Extended Sensitivity and Range

Examining microbial growth characteristics is one of the most fundamental studies in general microbiology. Typically, bacterial growth studies are accomplished by measuring optical density (O.D.) of the microbial culture

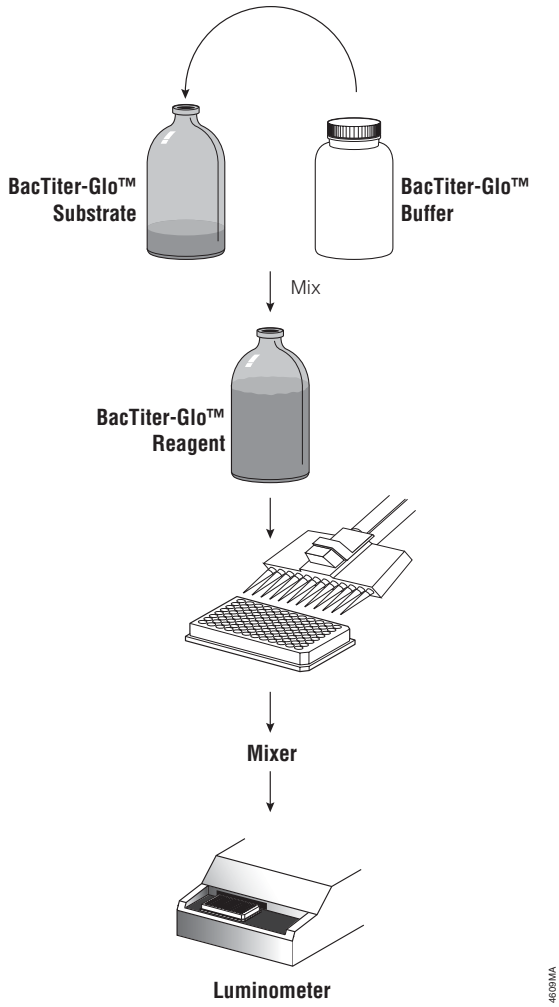


Figure 1. Diagram of the BacTiter-Glo™ Microbial Cell Viability Assay protocol. The assay is suitable for multiwell-plate assays for high-throughput screening.

at ~600nm. Here, we compare the BacTiter-Glo™ Assay to O.D. measurement for examining the growth of *E. coli* (Figure 2). The extended sensitivity and range of the BacTiter-Glo™ Assay allows us to monitor growth of *E. coli* immediately after inoculation. In contrast, the first significant change in $O.D._{600}$ measurement (0.025) does not occur until 5 hours after inoculation.

The growth curve determined by the BacTiter-Glo™ Assay has a dynamic range over six orders of magnitude compared to the growth curve determined by $O.D._{600}$ measurement, which only has a range of about two orders of magnitude. The growth rates measured by both methods are comparable. Furthermore, the add-mix-

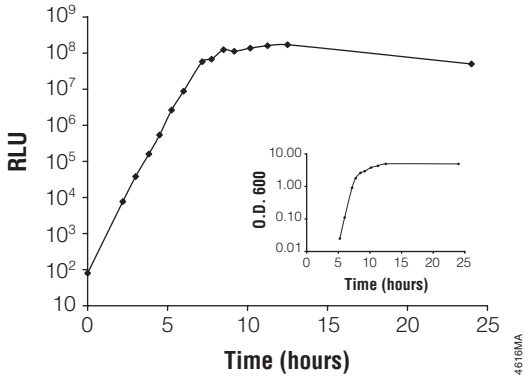


Figure 2. Comparison of growth curves obtained using the BacTiter-Glo™ Assay and by optical density measurement. *E. coli* ATCC 25922 strain was grown in Mueller Hinton II (MH II) broth (B.D. Cat.# 297963) at 37°C overnight. The overnight culture was diluted 1:10^6 in 50ml of fresh MH II broth and incubated at 37°C with shaking at 250rpm. Samples were taken at various time points, and the BacTiter-Glo™ Assay was performed according to the protocol described in Technical Bulletin #TB337. Luminescence was recorded on a Veritas™ Microplate Luminometer (Cat.# E6501). Optical density was measured at 600nm ($O.D._{600}$) using a Beckman DU650 spectrophotometer. Diluted samples were used when readings of relative light units (RLU) and O.D. exceeded 10^8 and 1, respectively.

measure format of the BacTiter-Glo™ Assay makes it as easy to perform as O.D. measurements. The increased dynamic range allows researchers to examine microbial growth at early time points and more easily monitor slow-growing bacteria.

Compatible with Multiwell Plates

The BacTiter-Glo™ Microbial Cell Viability Assay generates a glow-type luminescent signal that has a half-life of about 30 minutes or greater depending on the microbe and medium (1). The signal stability makes this assay amenable to applications in 96- or 384-well plates.

To assess this capability, we determined the Z′-factor value in each of these assay formats. Z′-factor is a statistical parameter of the assay (2). Values closer to 1.0 indicate higher precision. The BacTiter-Glo™ Assay has Z′-factor values of 0.90 and 0.87 for 96-well and 384-well formats, respectively (Figure 3). These experiments were performed using 10^6 cells/well, which is typical for conducting antimicrobial screening (3).

Screen Antimicrobial Compounds

We used the BacTiter-Glo™ Assay to screen a panel from the Library of Pharmacologically Active Compounds from Sigma (LOPAC, #8, enzyme inhibitors, total of 80 compounds) for antimicrobial activity against *Staphylococcus aureus* (Figure 4). All positive controls of standard antibiotics (boxed points) and three LOPAC compounds (circled points) exhibited significant anti-*S. aureus* activity. The three hits (emodin; sanguinarine chloride and minocycline) have been reported in the literature as having anti-*S. aureus* activities (4–6).

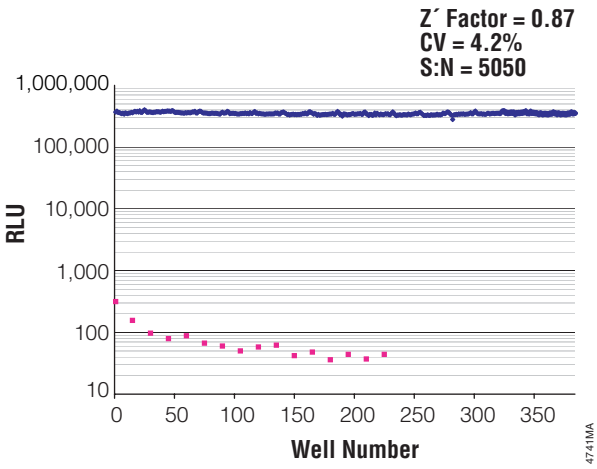


Figure 3. Scattergram of BacTiter-Glo™ Assay performed in a 384-well plate. *E. coli* (ATCC 25922) was grown in Mueller Hinton II (MH II) broth at 37°C overnight. The overnight culture was diluted 100-fold in fresh MH II broth and grown to log phase. The sample was diluted 1:10, and 25µl was dispensed in a 384-well plate using the Beckman Coulter Biomek® FX workstation. Reagent was added to the plate in equal volume to the sample, and luminescence was measured using the PHERAstar from BMG Laboratories at 0.5 seconds per well. Wells contained either 1 × 10^6 *E. coli* cells (blue) or no cells (pink) for measuring background luminescence.

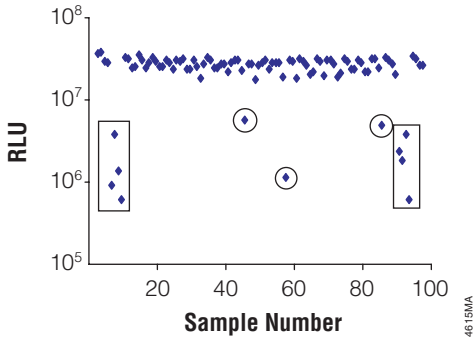
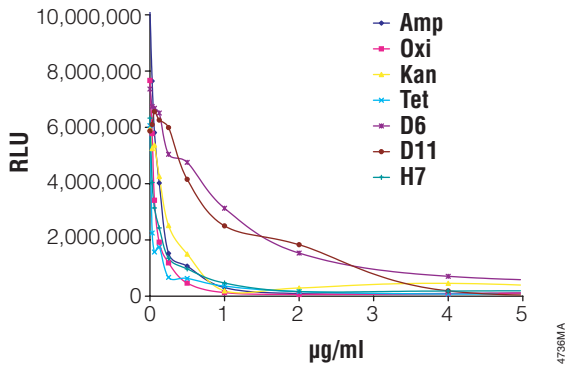


Figure 4. Screening for antimicrobial compounds using the BacTiter-Glo™ Assay. *S. aureus* ATCC 25923 strain was grown in Mueller Hinton II (MH II) Broth (BD Cat.# 297963) at 37°C overnight. The overnight culture was diluted 100-fold in fresh MH II Broth and used as inoculum for the antimicrobial screen. Working stocks (50X) of LOPAC compounds and standard antibiotics were prepared in DMSO. Each well of the 96-well plate contained 245µl of the inoculum and 5µl of the 50X working stock. The multiwell plate was incubated at 37°C for 5 hours. One hundred microliters of the culture was taken from each well, and the BacTiter-Glo™ Assay was performed according to the protocol described in Technical Bulletin #TB337. Luminescence was measured using a Veritas™ Microplate Luminometer (Cat.# E6501). The samples and concentrations are: wells 1–4 and 93–96, negative control of 2% DMSO; wells 5–8 and 89–92, positive controls of 32µg/ml standard antibiotics tetracycline, ampicillin, gentamicin, chloramphenicol, oxacillin, kanamycin, piperacillin and erythromycin; wells 9–88, LOPAC compounds at 10µM.

Evaluate Antimicrobial Compounds

We further examined the dosage effects of standard antibiotics and the three LOPAC hits on *S. aureus* using the BacTiter-Glo™ Assay and compared these with minimal inhibitory concentration (MIC) values determined by following the NCCLS protocol (3). All tested samples showed anti-*S. aureus* activity in a dosage-dependent fashion (Figure 5). The anti-*S. aureus* activities measured



MIC µg/ml	Amp	Oxa	Kan	Tet	D6	D11	H7
	1	0.5	1	1	4	4	1

Figure 5. Evaluating antimicrobial compounds using the BacTiter-Glo™ Assay. *S. aureus* ATCC 25923 strain and antibiotics were prepared as described in Figure 4 and incubated at 37°C; the assay was performed after 19 hours of incubation as recommended for MIC determination by NCCLS (3). Luminescence was recorded on a Veritas™ Microplate Luminometer (Cat.# E6501). D6, emodin; D11, sanguinarine chloride; H7, minocycline.

by the BacTiter-Glo™ Assay not only correlated well with the MIC values but also were more quantitative.

Conclusions

The new BacTiter-Glo™ Microbial Cell Viability Assay is a simple yet highly sensitive method for quantitating viable microbial cells. It is the only homogeneous single-step assay of its kind. The add-mix-measure format and stable luminescent signal of this assay are well suited for applications in multiwell plates. Moreover, the assay is an ideal choice for automation and high-throughput applications such as antimicrobial drug screening.

References

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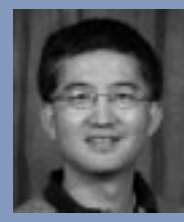

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Protocol

- *BacTiter-Glo™ Microbial Cell Viability Assay Technical Bulletin* #TB337, Promega Corporation.
  www.promega.com/tbs/tb337/tb337.html

Ordering Information

Product	Size	Cat.#
BacTiter-Glo™ Microbial Cell Viability Assay*	10ml	G8230
	10 × 10ml	G8231
	100ml	G8232
	10 × 100ml	G8233
Veritas™ Microplate Luminometer	1 each	E6501
20/20^{n} Luminometry System	1 each	E5311

*For Laboratory Use.

[a] U.S. Pat. No. 6,602,677, Australian Pat. No. 754312 and other patents pending.

[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.