Microbial Detection That Glows

Quantitate Microbial Cells Using a Rapid and Sensitive ATP-Based Luminescent Assay

By Frank Fan, Ph.D., Braeden Butler, B.S., Terry Riss, Ph.D., and Keith Wood, Ph.D., Promega Corporation

Abstract

The BacTiter-Glo™ Microbial Cell Viability Assay determines the number of viable cells based on quantitation of 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. You can easily adapt the assay to single-tube or multiwell formats, and you can measure luminescence using either a luminometer without injectors or CCD camera.

The "add, mix and measure" format of this assay makes it an ideal choice for both antimicrobial drug discovery and general microbiology.

Introduction

ATP-based detection and quantitation of microbial cells represents a key application of luciferase/luciferin bioluminescence technology. Conventional methods require two steps: addition of a lysis reagent to release microbial ATP, followed by a detection reagent to elicit bioluminescence. We have developed the BacTiter-Glo™ Microbial Cell Viability Assay[a,b], which combines the lytic reagent with luciferase/luciferin and allows sensitive detection of microbial cells in a single step.

The assay system utilizes a proprietary reagent formulation containing a thermostable luciferase, Ultra-Glo™ Recombinant Luciferase, to simultaneously extract ATP from bacterial cells and support a stable "glow-type" luminescent signal. Historically, firefly luciferase purified from *Photinus pyralis* has been used in reagents for ATP assays (1–3). However, this enzyme has only moderate stability in vitro and is sensitive to factors such as pH and detergents, limiting its usefulness in a robust and homogeneous ATP assay. We developed a stable form of luciferase based on the gene from another firefly, *Photuris pennsylvanica*, using an approach to select for characteristics that improve performance in ATP assays (4). In addition, we developed a proprietary formulation to achieve rapid and more efficient extraction of ATP from a variety of microbial cells. The combination of these two essential elements in the BacTiter-Glo™ Assay enabled the design of a homogeneous, single-reagent system for performing ATP assays on cultured cells. The reagent is physically robust and provides a sensitive and stable luminescent output.

Simply Add, Mix and Measure

The assay is simple to use and requires only a single addition of reagent directly to cells in the culture medium. The procedure does not require additional handling steps, such as removing medium or washing cells, thus reducing errors that may be introduced during a multiple-step procedure. The stable signal eliminates the need for injectors.

A diagram of the assay protocol is shown in Figure 1. To prepare BacTiter-Glo™ Reagent, the lyophilized BacTiter-Glo™ Substrate is reconstituted using BacTiter-Glo™ Buffer and equilibrated at room temperature for at least 15 minutes to reduce background luminescence (see Technical Bulletin #TB337 for details). To perform the assay, an equal volume of reagent is added to the microbial cell culture, mixed and incubated for 5 minutes. The emitted luminescence is detected using a luminometer or a charged coupled device (CCD) camera.

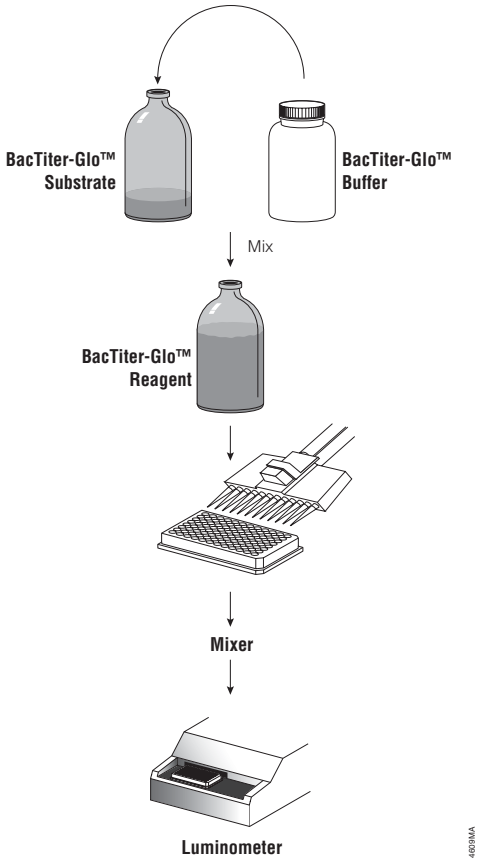

Figure 1. Diagram of the BacTiter-Glo™ Microbial Cell Viability Assay protocol. The assay is suitable for the multiwell plate format shown here as well as single-tube assays.

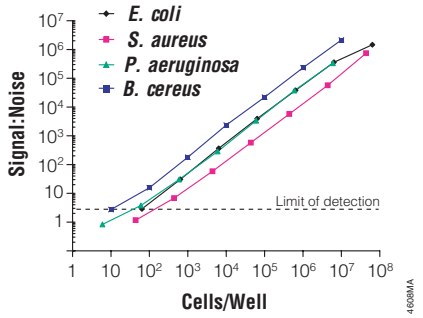

Figure 2. Bacterial cell numbers correlate with luminescent signal. Four bacterial strains *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC25923), *Pseudomonas aeruginosa* (ATCC27853) and *Bacillus cereus* (ATCC10987) were grown in Mueller Hinton II (MH II) Broth (see Technical Bulletin #TB337 for growth medium recommendations) overnight at 37°C. The overnight culture was diluted 50-fold in fresh MH II Broth and then incubated for several hours to reach log phase. Samples of the culture were serially diluted using MH II Broth in a 96-well plate. The assay was performed according to the protocol described in Technical Bulletin #TB337. The reconstituted BacTiter-Glo™ Reagent was equilibrated for 1.5 hours at room temperature to achieve increased sensitivity. Luminescence was recorded on a Veritas™ Microplate Luminometer (Cat.# E6501). Signal values represent the mean of three replicates for each measurement. Bacterial cell numbers were determined by plate counting of colony forming units on Luria-Bertani (LB) agar plates. The signal-to-noise ratio was calculated: S:N = [mean of signal–mean of background]/standard deviation of background]. The limits of detection from this experiment for *E. coli*, *S. aureus*, *P. aeruginosa* and *B. cereus* are approximately 40, 150, 70 and 10 cells, respectively.

Detect as Few as Ten Cells

The BacTiter-Glo™ Microbial Cell Viability Assay is sensitive and linear (Figure 2). The data in Figure 2 demonstrate that the assay can detect as few as 10 *Bacillus cereus* cells, which gave signal levels greater than three standard deviations above the background signal from medium without cells. Luminescent signals correlated well with the cell numbers, with excellent linearity with a dynamic range typically over 5 logs. The extended sensitivity and range of the BacTiter-Glo™ Assay also allows users to monitor *E. coli* growth immediately after inoculation (see New Products Section).

Gain Flexibility With Stable Signal

The BacTiter-Glo™ Microbial Cell Viability Assay generates a glow-type luminescent signal that has a half-life generally over 30 minutes depending on the microbe and medium (Figure 3). High sensitivity and signal stability make the BacTiter-Glo™ Assay highly amenable for high-throughput screening. This is reflected by excellent Z´-factor values. (Z´-factor is a measure of assay quality based on the dynamic range and data variability, 5). The BacTiter-Glo™ Assay has Z´-factor values of 0.90 and 0.87 for 96-well and 384-well formats, respectively.

Use with Typical Media and Solvents

The chemical environment of the luciferase reaction will affect the enzymatic rate and thus luminescence intensity and kinetics. We have demonstrated that the BacTiter-Glo™ Assay is compatible with typical microbial growth

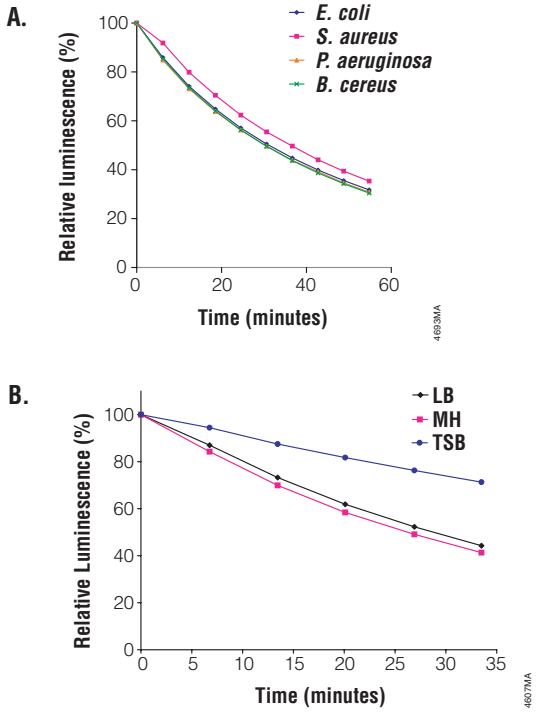

Figure 3. BacTiter-Glo™ Assay generates a glow-type luminescent signal. Bacterial cells were grown and assayed as described in Figure 2. Approximately 10^6 cells were used in each assay. The stability of the luminescent signal was monitored over time. Luminescence was recorded on a Veritas™ Microplate Luminometer (Cat.# E6501). **Panel A.** Four different bacteria: *E. coli*, *S. aureus*, *P. aeruginosa* and *B. cereus* were grown in Mueller Hinton II Broth and assayed. **Panel B.** *E. coli* cells were grown in three media: Luria-Bertani (LB), Mueller Hinton II Broth (MH) and Trypticase Soy Broth (TSB), and assayed.

media and commonly used organic solvents (Figure 4). In general, we recommend cation-adjusted Mueller Hinton broth (MH II Broth, BD Cat.# 297963). It supports growth for most commonly encountered aerobic and facultative anaerobic bacteria and has been selected for use in food testing and antimicrobial susceptibility testing by the Food and Drug Administration (FDA) and the National Committee for Clinical Laboratory Standards (NCCLS) (6,7). In our hands, this medium has low luminescence background and good batch-to-batch reproducibility. Dimethylsulfoxide (DMSO), commonly used as a vehicle to solubilize organic chemicals, had little effect on luminescent signal when tested at a final concentration of 3%.

Test a Variety of Microbial Organisms

We have demonstrated the utility of the BacTiter-Glo™ Assay for detecting and quantitating a variety of microbes as shown in Table 1. These microbes include Gram-positive and Gram-negative bacteria, yeast and fungi. They also include some common pathogens of interest for antimicrobial drug discovery, biothreat pathogen surrogates of interest for biodefense, model microbial organisms as well as some random collections.

Microbial Cell Viability Assay...continued

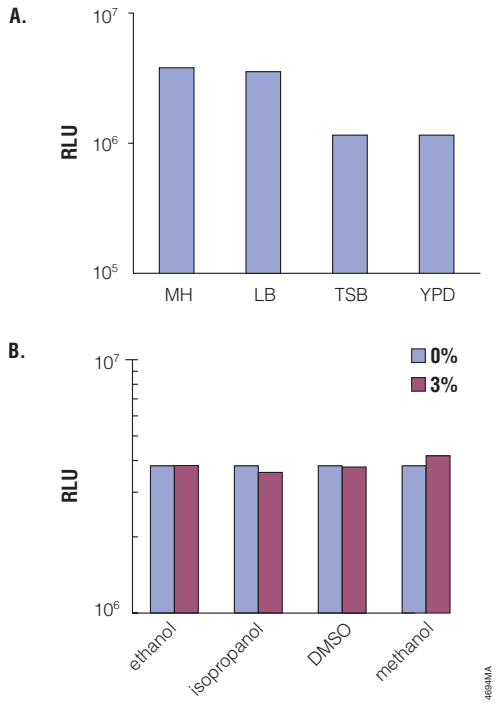

Figure 4. BacTiter-Glo™ Assay is compatible with typical media and solvents. Approximately 1×10^{-12} mole of ATP was used in each assay. Luminescence was recorded on a Veritas™ Microplate Luminometer (Cat.# E6501). **Panel A.** Luminescence was measured from samples in four different media: Mueller Hinton II Broth (MH), Luria-Bertani (LB), Trypticase Soy Broth (TSB) or Yeast Peptone Dextrose (YPD). **Panel B.** Luminescence was measured from samples of MH medium with either 0% or 3% solvent.

Conclusions

The new BacTiter-Glo™ Microbial Cell Viability Assay is a simple, yet highly sensitive method for quantitation and detection of viable microbial cells. The assay uses a single reagent to release and measure ATP from microbial cells. It is the only homogeneous single-step assay of its kind. The "add, mix and measure" format of this assay makes it an ideal choice for both antimicrobial drug discovery and general microbiology.

References

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Table 1. Organisms Successfully Tested with the BacTiter-Glo™ Assay.

Gram– Bacteria	Gram+ Bacteria	Others
Escherichia coli[1]	*Staphylococcus aureus*[2]	*Saccharomyces cerevisiae*[1]
Psuedomonas aeruginosa[2]	*Enterococcus faecalis*[2]	*Candida albicans*[2]
Enterobacter cloacae	*Streptococcus pneumoniae*[2]	
Flavobacterium okeanokoites	*Bacillus subtilis*[1]	
Haemophilus influenzae[2]	*Bacillus cereus*[3]	
Proteus vulgaris	*Arthrobacter luteus*	
Salmonella typhimurium		
Yersinia enterocolitica[3]		
Francisella philomiragia[3]		

[1]*Model organisms*
[2]*Drug discovery*
[3]*Biodefense*

Protocol

- *BacTiter-Glo™ Microbial Cell Viability Assay Technical Bulletin* #TB337, Promega Corporation. (www.promega.com/tbs/tb337/tb377.html)

Frank Fan, Ph.D.
Research Scientist

Braeden Butler, B.S.
Research Scientist

Terry Riss, Ph.D.
Research Scientist

Keith Wood, Ph.D.
Research Fellow

Ordering Information

Product	Size	Cat.#
BacTiter-Glo™ Microbial Cell Viability Assay	10ml	G8230
	10 × 10ml	G8231
	100ml	G8232
	10 × 100ml	G8233

For Laboratory Use.

[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] 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.