

A Homogenous Bioluminescent Assay for High-throughput Antimicrobial Screening

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Abstract

ATP-based detection of microbial cells represents a key application of luciferase/luciferin bioluminescence assay. Conventional methods require two steps: application of a lysis reagent to release microbial ATP, followed by a detection reagent to elicit bioluminescence. We have developed an assay that combines the lytic reagent with luciferase/luciferin, thus allowing sensitive detection of microbial cells in a single-step. The assay system utilizes a thermostable luciferase to enable extraction of ATP from bacterial cells and to support a stable "glow-type" luminescent signal. In addition, we developed a formulation to achieve rapid and efficient extraction of ATP from a variety of microbial cells. The combination of these two essential elements enabled the design of a homogeneous single-reagent system (BacTiter-Glo™ Reagent) for performing ATP assays on cultured cells.

Discovery and development of new antibiotics are in critical demand to combat infectious diseases caused by microbial pathogens and rapid spreading of antibiotic resistance. The "add-mix-measure" format of the BacTiter-Glo™ Assay can be easily adapted for high-throughput screening. In this report, we describe the use of BacTiter-Glo™ Assay to screen and evaluate antimicrobial compounds.

Half-life

Assay Allows Rapid Extraction of Microbial ATP Luminescence Stable with a Half-life > 30 Minutes

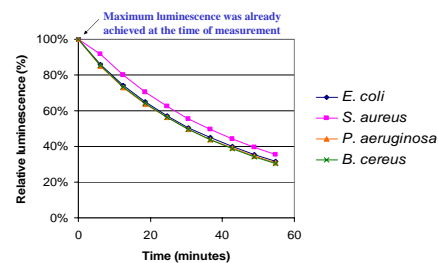


Figure 2. The half-life of luminescent signal produced by BacTiter-Glo™ Assay is typically greater than 30 minutes, thus enabling high throughput analysis of samples. Luminescent signal was measured in triplicate of log-phase cultures.

Partial List of Compatible Microbes*

Gram- bacterium	Gram+ bacterium	Others
<i>Escherichia coli</i> ¹	<i>Staphylococcus aureus</i> ²	<i>Saccharomyces cerevisiae</i> ¹
<i>Pseudomonas aeruginosa</i> ²	<i>Enterococcus faecalis</i> ²	<i>Candida albicans</i> ²
<i>Enterobacter cloacae</i>	<i>Streptococcus pneumoniae</i> ²	
<i>Flavobacterium okeanoicoles</i>	<i>Bacillus subtilis</i> ¹	
<i>Haemophilus influenzae</i> ²	<i>Bacillus cereus</i> ³	
<i>Proteus vulgaris</i>	<i>Arthrobacter luteus</i>	
<i>Salmonella typhimurium</i>		
<i>Yersinia enterocolitica</i> ³		
<i>Francisella philomiragia</i> ³		

*These are the microbes we have tested so far.

¹: Model organism

²: Drug discovery

³: Biodefense

Antimicrobial Susceptibility Testing

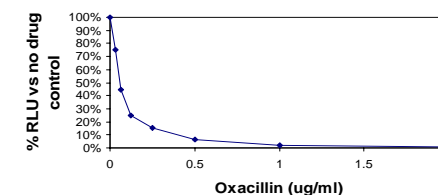
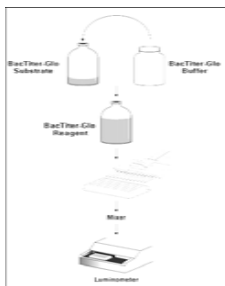


Figure 7. Dosage effects of oxacillin on *S. aureus* using the BacTiter-Glo™ Assay were examined. Oxacillin showed anti-*S. aureus* activity in a dosage dependent fashion. The reported and observed MIC values for oxacillin on *S. aureus* ATCC25923 in cation-adjusted Mueller Hinton broth are 0.125-0.5 ug/ml, corresponding to approximately IC₇₅-IC₉₀ values on the dosage curve determined using BacTiter-Glo™ Assay.

Homogeneous Method

BacTiter-Glo™ Assay Protocol



- Mix to form BacTiter-Glo™ Reagent
 - Equilibrate for ≥15 min
 - Add 100 µl of reagent with 100 µl of cells
 - Mix and incubate for 5 minutes
 - Measure luminescence
- (All steps performed at room temperature)

Compatible with Standard Media and Solvents

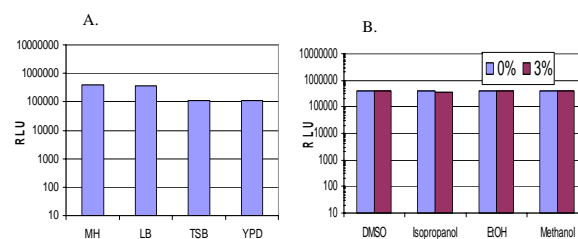


Figure 3. BacTiter-Glo™ Assay composition has little interference for ATP quantitation from either (A) media variations and (B) from common solvents for test compounds MH: Mueller Hinton; LB: Luria-Bertani; TSB: Trypticase Soy Broth, YPD: Yeast Extract-Peptone-Dextrose.

Screening for Antimicrobial Compounds

Allows Sensitive Identification of Hit Compounds at Early Time Point

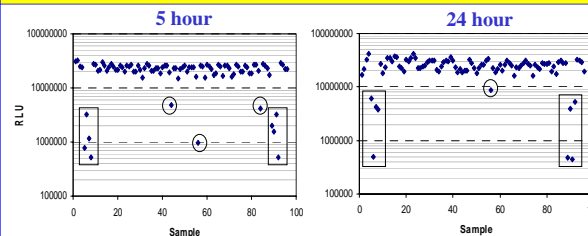


Figure 5. BacTiter-Glo™ Assay was used to screen some LOPAC compounds (Rack #8) from Sigma for the antimicrobial activity against *Staphylococcus aureus*. All positive controls of standard antibiotics (boxed points) and three compounds (circled points) exhibited significant anti-*S. aureus* activity. The three hits were emodin, sanguinarine chloride and minocycline. Their anti-*S. aureus* activities were more easily identified with 5 hr incubation than with 24 hr incubation.

Summary

BacTiter-Glo™ Assay is Ideal for HTS of Antimicrobial Compounds

- Homogeneous, single-step assay reagent
- Stable signal with half-life typically greater than 30 minutes
- Data recorded 5 min or shorter after mixing
- Sensitive detection as low as 10 bacterial cells with extended dynamic range typically over 5 logs
- Compatible with a variety of media and solvents
- Works on a variety of microbial organisms

Sensitivity and Linearity

Detect as Low as 10 Bacterial Cells Linear Over Five Orders of Magnitude

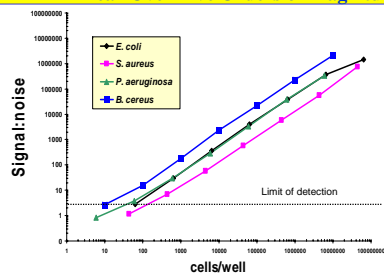


Figure 1. The correlation between luminescence and the number of cells is linear over five orders of magnitude. The limit of detection (S:N = 3) for *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus cereus* are approximately 40, 150, 70 and 10 cells, respectively. Calculation of signal-to-noise ratio: S:N = (mean of measurement - mean of background)/(standard deviation of background)

Suitable for High-throughput Screening

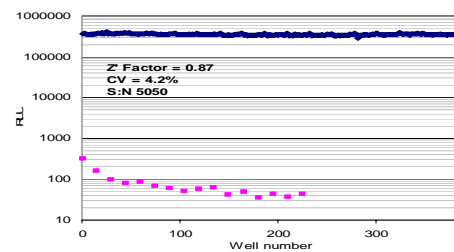


Figure 4. Scattergram of BacTiter-Glo™ Assay performed in a 384-well plate containing 1 x 10⁶ *E. coli* cells, or without cells for measuring background luminescence. The signal was consistent across the entire plate and CV, Z' and signal to noise ratios were within acceptable ranges for screening.

Evaluation of Antimicrobial Compounds

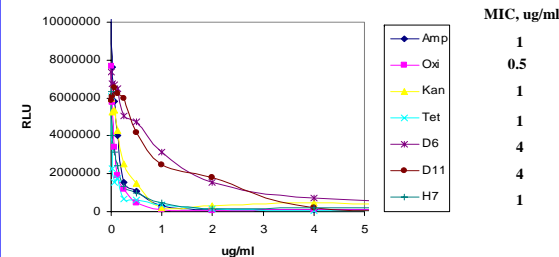


Figure 6. Dosage effects of standard antibiotics and LOPAC hits on *S. aureus* using the BacTiter-Glo™ Assay were examined and compared with MIC values determined by following the NCCLS protocol. All tested samples showed anti-*S. aureus* activity in a dosage dependent fashion. The anti-*S. aureus* activities measured by BacTiter-Glo™ Assay correlated well with the MIC values.

For additional information, see <http://www.promega.com/tbs/tb337>