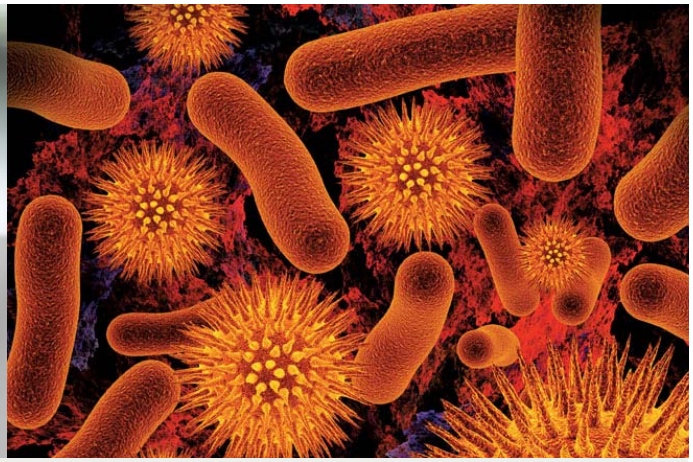

Determination of microbial cell viability

BacTiter-Glo™ Microbial Cell Viability Assay measured on the Infinite® 200 PRO multimode microplate reader

Introduction

The BacTiter-Glo Microbial Cell Viability Assay is a luminescence-based assay for the determination of the viability of microbial cells, using quantification of ATP as an indicator of metabolically active, viable cells (1). The chemistry relies on the properties of a thermostable luciferase (Ultra-Glo™ Recombinant Luciferase, Figure 1) and a proprietary formulation for extracting ATP from bacteria. The assay protocol comprises the addition of the BacTiter-Glo reagent directly to the sample, and subsequent measurement of luminescence; cell washing, removal of culture medium, and multiple pipetting steps are not required (Figure 2). The luminescent signal generated is proportional to the amount of ATP, and therefore an indicator of the number of viable cells in the sample. The 'glow-type' nature of the assay permits signal measurement over a period of approximately 30 minutes, depending on the type of bacteria and growth medium.

The BacTiter-Glo Microbial Cell Viability Assay was tested on the Infinite F200 PRO filter-based multimode reader, using the instrument's highly sensitive luminescence module.

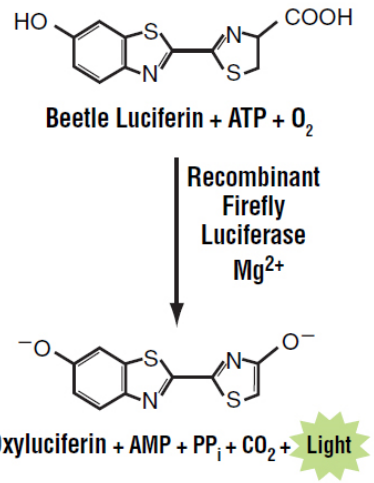

Figure 1 The luciferase reaction (1, copied with the permission of Promega Corp.).

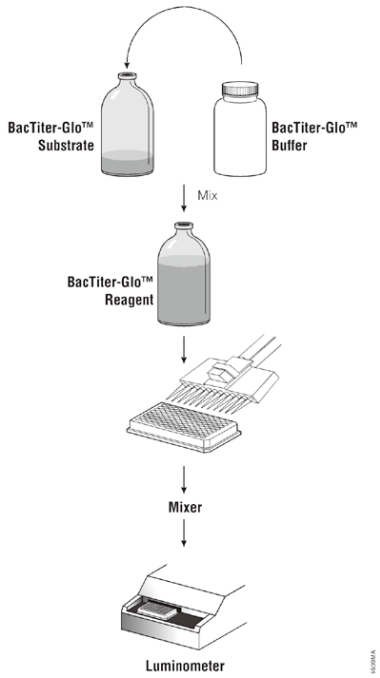

Figure 2 Schematic of the BacTiter-Glo Microbial Cell Viability Assay protocol (1, copied with the permission of Promega Corp.).

Materials and methods

- Infinite F200 PRO filter-based multimode reader (Tecan, Austria)
- 96-well, white polystyrol microplate (Greiner®, Germany)
- BacTiter-Glo Microbial Cell Viability Assay kit (Promega, USA)

BacTiter-Glo Microbial Cell Viability Assay reagents were prepared according to the assay instructions (1). To validate the assay, a simple ATP dilution curve was prepared according to the assay instructions (1), and measured on the Infinite F200 PRO using the instrument settings shown in Table 1. Furthermore, the assay was used to detect the bacterial burden of publicly accessible locations and commonplace items, including:

- Computer mouse
- Laboratory floor
- Toilet water
- Tap water
- Dish washing sponge
- Laminar flow hood bench (for cell culture)

Samples were taken from the computer mouse, the laboratory floor and the laminar flow hood bench using sterile cotton buds, which were then transferred to an Eppendorf tube containing 500 µl ATP-free H_2O. Samples (~500 µl) were taken directly from toilet water, tap water and the dish washing sponge. The assay was performed according to the manufacturers' instructions, with an incubation time of 15 minutes for both the ATP dilution series and samples.

Measurement parameter	Instrument settings
Plate	GRE384sw.pdfx
Mode	Luminescence
Attenuation	Automatic
Integration time	1,000 ms
Settle time	0 ms

Table 1 Infinite 200 PRO measurement parameters and instrument settings for the BacTiter-Glo Microbial Cell Viability Assay.

Results and discussion

The ATP dilution curve determined on the Infinite F200 PRO shows perfect linearity over five orders of magnitude (Figure 3).

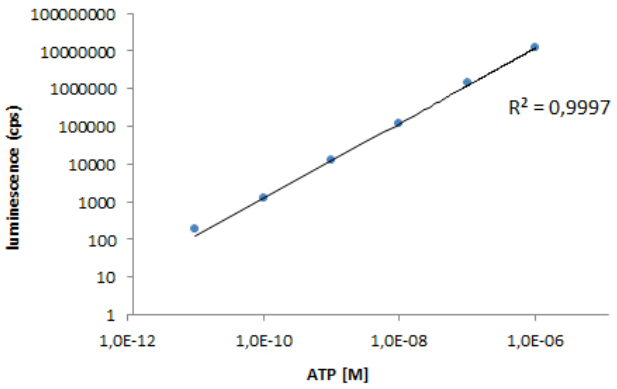

Figure 3 ATP dilution curve.

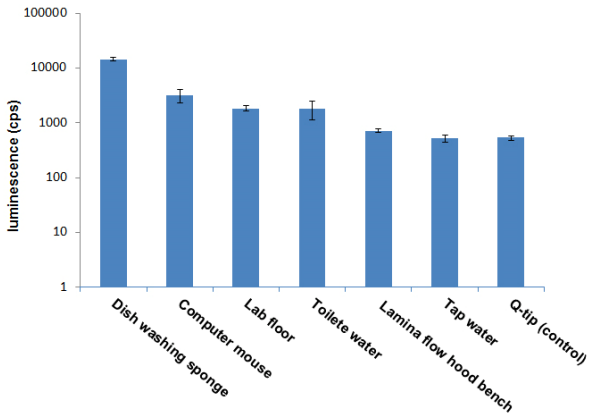

Figure 4 Bacterial burden determined using the BacTiter-Glo Microbial Cell Viability Assay and the Infinite F200 PRO reader.

Figure 4 shows the bacterial burden of some selected publicly accessible locations and commonplace items. As expected, the dish washing sponge, computer mouse, laboratory floor, and toilet water showed a significant level of bacterial contamination, whereas the cell culture lamina flow hood bench and the tap water remained at control level.

Conclusion

This study clearly demonstrates the compatibility of Tecan's Infinite F200 PRO filter-based multimode microplate reader with Promega's BacTiter-Glo Microbial Cell Viability Assay, enabling the determination of the bacterial burden of various materials. The ATP dilution curve determined shows perfect linearity over a dynamic range of more than five orders of magnitude.

Literature

1) Technical Bulletin: BacTiter-Glo Microbial Cell Viability Assay. Instructions for use of products G8230, G8231, G8232 and G8233, part# TB337, Promega.

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