BacTiter-Glo™ Microbial Cell Viability Assay

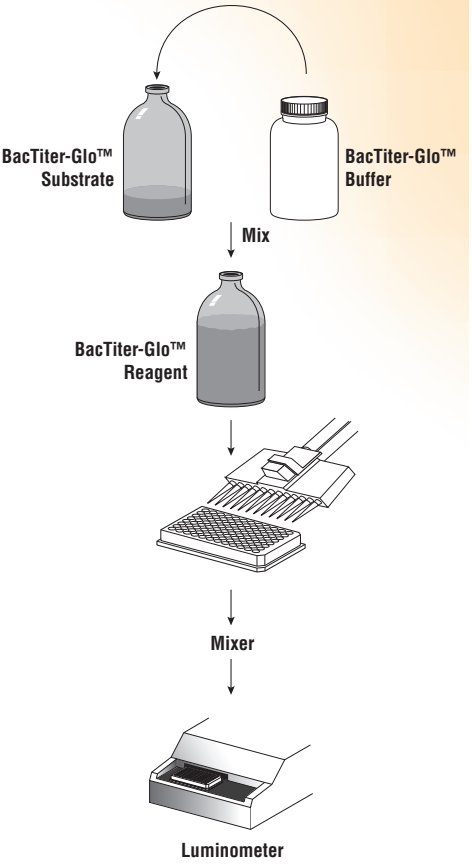
INSTRUCTIONS FOR USE OF PRODUCTS G8230, G8231, G8232 AND G8233.

Quick PROTOCOL

Protocol for Measuring ATP from Bacteria

Before beginning the protocol, see **Section 3.A., Reagent Preparation**, in the *BacTiter-Glo™ Microbial Cell Viability Assay Technical Bulletin*, #TB337.

1. Perform the following steps at room temperature (22°–25°C).
2. Prepare an opaque-walled multiwell plate with microbial cells in culture medium (e.g., 100µl/well for 96-well plate or 25µl/well for 384-well plate).
3. Equilibrate this plate and contents to room temperature.
4. Add a volume of BacTiter-Glo™ Reagent equal to the volume of cell culture medium in each well (e.g., 100µl/well for 96-well plate or 25µl/well for 384-well plate).
5. Mix the plate contents briefly on an orbital shaker and incubate for 5 minutes.
6. Read the plate and record luminescence.



Generating a Standard Curve (Optional)

1. Perform the following steps at room temperature (22°–25°C).
2. Prepare 1µM ATP in culture medium (100µl of ATP solution contains 10^{-10} moles of ATP).
3. Prepare 10-fold serial dilutions of ATP in culture medium (1µM to 10pM; 100µl volumes would contain 10^{-10} to 10^{-15} moles of ATP).
4. Prepare a multiwell plate with varying concentrations of standard ATP solution in 100µl of medium.
5. Add a volume of BacTiter-Glo™ Reagent per well equal to the volume of ATP standard present in each well (a 1:1 ratio).
6. Mix contents briefly on an orbital shaker and incubate for 1 minute. No lysis is required to release ATP, so longer incubations are not required.
7. Read the plate and record luminescence.

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