Measuring ATP in Low Bioburden Water Samples Using the Water-Glo™ Kit

Promega Corporation

Kit:
Water-Glo™ Microbial Testing Kit, 20 Single Tests (Reagents Only; Cat.# AM1002)
Includes:

- Water-Glo™ Buffer, 1 × 10ml
- Water-Glo™ Substrate, 1 vial
- Water-Glo™ Lysis Reagent, 2 × 65ml
- Water-Glo™ ATP Standard, 1 × 2ml

Storage of Reagents:
All components can be stored at room temperature (20–25°C) prior to initial use. The reconstituted Water-Glo™ Detection Reagent can be stored at 4°C for 6 months or at room temperature (23°C) for two weeks without significant loss of performance. All other components can be stored at room temperature after initial use.

Materials Required:

- Water-Glo™ Microbial Testing Kit, 20 Single Tests (Reagents Only; Cat.# AM1002)
- Corning® 50ml Tube Top Vacuum Filter System, 0.22µm Pore 13.6cm² CA Membrane, Sterile (Corning Cat.# 430320)
- Nalgene™ Rapid-Flow™ Sterile Filter Storage Bottle, 1L (Thermo Scientific Cat.# 455-1000 or Fisher Scientific Cat.# 09-740-25F)
- 1.5ml Microfuge Tubes
- GloMax® 20/20 Luminometer (Cat.# E5311)
- Welch® Vacuum Pump (Cat.# A6720 for North America electrical, Cat.# A6722 for European electrical, Cat.# A6724 for Japanese electrical)

Introduction

Water used for manufacturing of consumer goods, beverages and pharmaceuticals must be tested for microbial contamination. Typically, such water has low bioburden. Traditional microbial compendial methods are based on culturing techniques which are labor-intensive and time-consuming, often requiring days of incubation. Therefore, production is seldom able to take proactive corrective action based on the results. In addition, culturing conditions may impact sensitivity, accuracy and reproducibility. Culture-independent rapid microbiological methods can overcome these challenges. One of these methods is based on detection of ATP, the cellular energy currency. Cellular ATP levels effectively correlate with viable biomass or metabolic activity.

This protocol is designed to measure ATP from water samples that have low bioburden using the Water-Glo™ Kit (Cat.# AM1002). The kit utilizes a proprietary thermostable luciferase system that generates light upon reacting with ATP (Figure 1). The luminescent signal is directly proportional to the amount of cellular ATP, which correlates with the amount of active biomass in the sample. This simple workflow allows flexibility in sample preparation, and the sensitive reagents are resistant to environmental factors (e.g., storage temperature, chemical inhibitors).

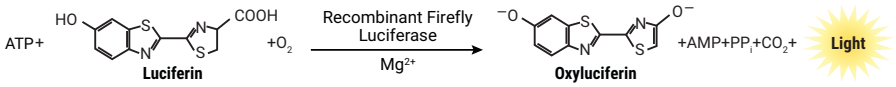

Figure 1. Schematic diagram of Water-Glo™ technology.

In this assay, filters are used to concentrate viable biomass from a 1 liter (L) water sample onto the filter surface. Small aqueous contaminants will pass through the filter, but biomass will not. This level of concentration allows microbial detection of more than 50 CFU/100ml. The captured biomass is extracted using the Water-Glo™ Lysis Reagent to release the cellular ATP. The Lysis Reagent is designed to extract cellular components from hard-to-lyse samples, including biofilms. The captured ATP is then detected using the Water-Glo™ Detection Reagent. For samples that contain an oil/water mixture, an additional organic wash step is used to remove inhibitory organic compounds from the filter. The Detection Reagent, which is made by reconstituting the Water-Glo™ Substrate using the Water-Glo™ Buffer, is added to the recovered lysate solution. The resulting light output is proportional to the amount of ATP present in the sample and can be measured with a luminometer (sensitivity can reach as low as 1pg/L ATP). 1pg of ATP is approximately equivalent to the amount of ATP present in 1,000 *E. coli*-sized bacteria in their exponential growth phase.

Protocol

1. Prepare the Water-Glo™ Detection Reagent by adding the Water-Glo™ Buffer to the lyophilized Water-Glo™ Substrate.
2. Detach the 50ml tube from the Corning® 50ml Tube Top Vacuum Filter System. Close the 50ml tube with the cap and keep it separate.
3. Attach the filter top to the mouth of the 1L Nalgene™ Rapid-Flow™ Sterile Filter Storage Bottle.
4. Assemble the filtration unit by connecting with a Welch® Vacuum Pump. Filter the 1L water sample by passing it through the vacuum filter and collecting flow-through in the 1L storage bottle. Take the filter top out of the storage bottle. Discard the water in the storage bottle. The storage bottle may be used for multiple filtrations to collect the flow-through.
5. Attach the 50ml tube to the filter top. Place the setup in the stand for the 50ml tube. Add 2ml of Water-Glo™ Lysis Reagent. Allow the Lysis Reagent to be in contact with the captured biomass on the filter for at least 1 minute.

   Note: If an extended lysis time is desired (e.g., potentially dealing with biofilms), pass a portion of the Lysis Reagent through the filter and incubate for up to 10 minutes. This allows increased contact time of the biomass with the Lysis Reagent. Once the incubation is complete, the remaining Lysis Reagent can be passed through the filter and collected in the 50ml collection tube.

   If you have multiple water samples, process all samples up to this step first. Then perform the ATP detection step for each sample.
6. Transfer 400µl of lysate into a clean 1.5ml microfuge tube, then add 400µl of Detection Reagent. Mix for 5 seconds and read the luminescence signal immediately using the GloMax® 20/20 Luminometer (use default setting). Record the RLU value (RLU_{sample}).
7. Positive Control: Add 400µl of the ATP Standard (1,000 pg/ml ATP) and 400µl of Detection Reagent into a clean 1.5ml centrifuge tube. Mix for 5 seconds and read the luminescence signal immediately using the GloMax® 20/20 Luminometer (use the default setting). Record the RLU value ($RLU_{ATP\ standard}$).

   Note: Do not dilute the Water-Glo™ ATP Standard in water. If a lower concentration is needed, dilute in Lysis Reagent.
8. Negative Control: Add 400µl of Lysis Reagent and 400µl of Detection Reagent into a clean 1.5ml microfuge tube. Mix for 5 seconds and read the luminescence signal immediately using the GloMax® 20/20 Luminometer. Record the RLU value ($RLU_{lysis\ reagent}$).
9. Measure the ATP content of the sample using the following formula:

$$ATP\ (pg/L) = \frac{RLU_{sample} - RLU_{lysis\ reagent}}{RLU_{ATP\ standard} - RLU_{lysis\ reagent}} \times \frac{V_{lysis\ reagent}}{V_{sample}} \times 1{,}000{,}000$$

$V_{lysis\ reagent} = 2ml$

$V_{sample} = 1{,}000ml$

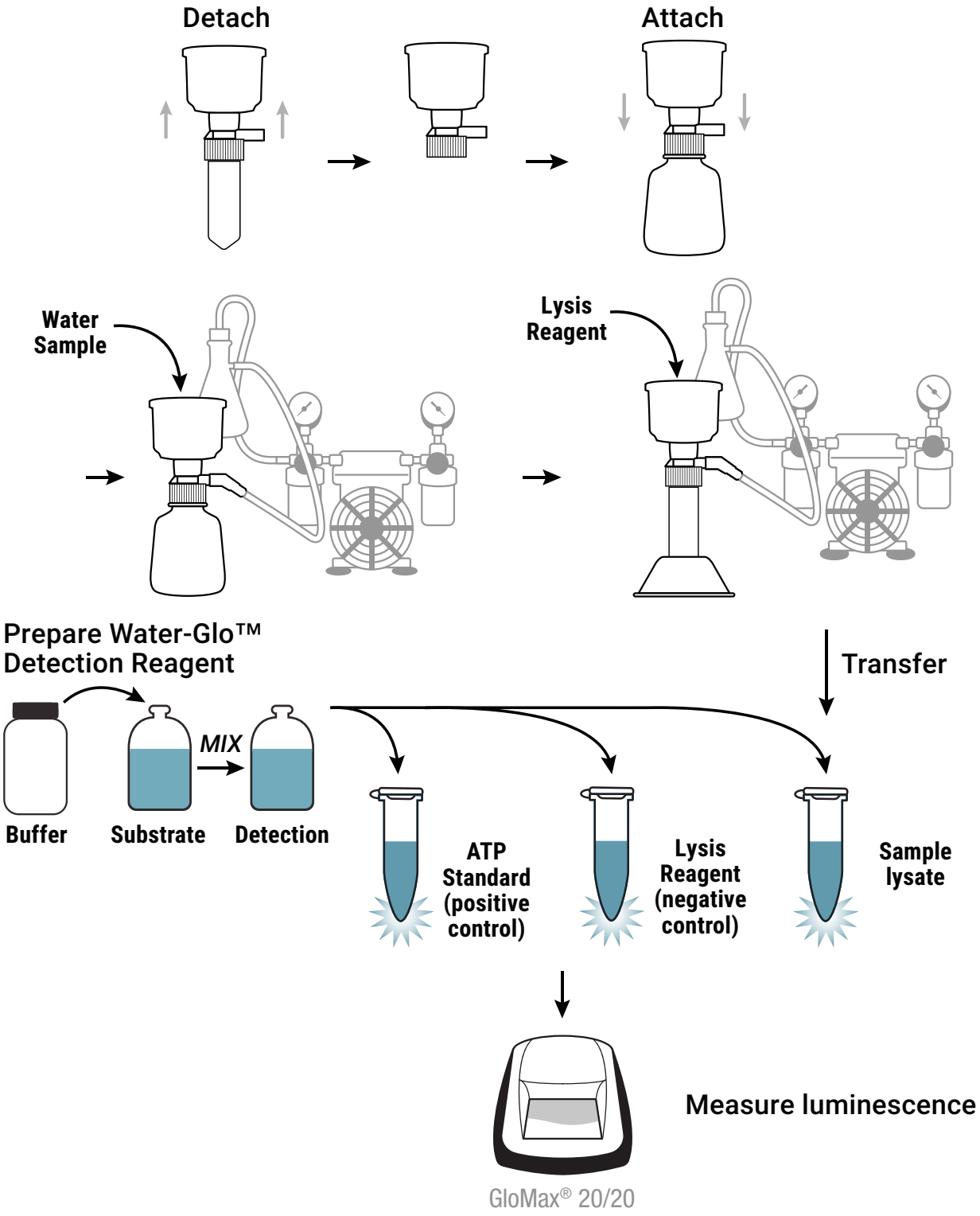

Figure 2. Water-Glo™ low bioburden protocol.

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