Water-Glo™ for Total Biocontamination Detection: Swabs, Solid Samples and Coupons

Promega Corporation

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

- Water-Glo™ Buffer, 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 Water Testing Kit, 20 Single Tests (Reagents Only; Cat.# AM1002)
- GloMax® 20/20 Luminometer (Cat.# E5311)

Materials Required (not provided in the kit):

- sterile swabs (e.g., Puritan 6" Sterile Standard Rayon Swab w/ Polystyrene Handle, SKU# 25-806 1PR)

Introduction

ATP levels in the environment effectively correlate with viable biomass or metabolic activity. Therefore, ATP levels can be used to monitor contamination and cleanliness, and guide the management of corrosion and biofilm formation. The Water-Glo™ Microbial Water Testing Kit, 20 Single Tests (Reagents Only; Cat.# AM1002) can be used to measure microbial content from surfaces, deposits and biofilm collection devices (including corrosion coupons).

The method utilizes the ATP-dependence of the firefly luciferase reaction to detect the presence of biological material (including microbial cells or food residues) on metal surfaces, deposits and biofilms. The light from fireflies is produced by an enzyme called luciferase, which catalyzes an ATP-dependent oxidation of luciferin. This reaction is shown below:

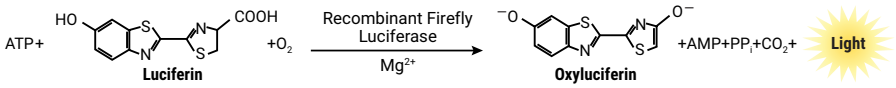

Figure 1. Schematic diagram of Water-Glo™ technology.

For measuring Total ATP from a surface, sample the surface using a sterile swab wetted in Water-Glo™ Lysis Reagent and then immerse the swab in the Lysis Reagent. Take a portion of the lysate and add the Water-Glo™ Detection Reagent. For measuring biocontamination in deposits and corrosion coupons, the sample is directly immersed in the Water-Glo™ Lysis Reagent and ATP is measured by adding the Water-Glo™ Detection Reagent. The light produced is proportional to the amount of ATP on the extracted sample, which is measured using a luminometer.

The Water-Glo™ Lysis Reagent is designed to extract ATP from hard-to-lyse sample types, including biofilms. For detection of ATP, we use a proprietary ATP detection reagent. The Water-Glo™ Detection Reagent is made by mixing the lyophilized substrate and Water-Glo™ Buffer. The reconstituted Detection Reagent is extremely stable, allowing flexible storage options. The reagent can be stored at 4°C for 6 months or at 23°C for two weeks with minimal loss in performance. All the other kit components are stable at ambient temperature for long periods of time.

Test Methods

Measurement of Total Biocontamination from Swabs

1. Reconstitute the Water-Glo™ Detection Reagent by adding the Water-Glo™ Buffer to the lyophilized Water-Glo™ Substrate.
2. Dispense 500µl of Water-Glo™ Lysis Reagent into a clean 1.5ml microfuge tube and label.
3. Open a sterile, unused swab and wet the swab tip with Lysis Reagent by immersing it in the 1.5ml microfuge tube containing the Lysis Reagent. **Wear gloves to prevent contamination. Do not touch the tip of the swab.**
4. Swab the area of interest (an area of 10cm × 10cm is recommended). Twist the swab between the fingers to maximize contact of the entire swab tip with the test area.
5. Place the swab back in the labeled 1.5ml microfuge tube containing the Lysis Reagent. Swirl the swab and let it sit in the Lysis Reagent for 1 minute for effective cell lysis.
6. Safely discard the swab.
7. Take 100µl of the lysate from the tube and put it in a new, clean 1.5ml microfuge tube, then add 100µl of the Water-Glo™ Detection Reagent.
8. Immediately read the sample luminescence using the GloMax® 20/20 Luminometer (RLU_{sample}).
9. Negative Control: Take 100µl Lysis Reagent and put it in a 1.5ml microfuge tube, then add 100µl of Water-Glo™ Detection Reagent. Immediately read the luminescence using GloMax® 20/20 ($RLU_{lysis\ reagent}$). This reading is used to determine if any of the reagents are contaminated.
10. Positive Control: Take another clean 1.5ml microfuge tube and add 100µl of the Water-Glo™ ATP Standard, then add 100µl of Water-Glo™ Detection Reagent. Immediately read the luminescence using the GloMax® 20/20 Luminometer ($RLU_{ATP\ standard}$).
11. Calculate the Total ATP using the formula below: This worksheet uses the following equation to calculate ATP concentration in water samples:

$$\text{Total ATP}(pg/cm^2)\ (\text{swab}) = \frac{RLU_{sample} - RLU_{lysis\ reagent}}{RLU_{ATP\ standard} - RLU_{lysis\ reagent}} \times \frac{500pg}{\text{Surface Area } (cm^2)}$$

Example: For a 10cm × 10cm swabbing area, the Surface Area = $100cm^2$

Measurement of Total Biocontamination from Solid Samples

1. Reconstitute the Water-Glo™ Detection Reagent by adding the Water-Glo™ Buffer to the lyophilized Water-Glo™ Substrate.
2. Obtain a solid sample (e.g., filter media, corrosion sample). Weigh 1g of the deposit. The sample can be dry or wet. Place the sample into a 15ml conical tube.
3. Add 5ml of Water-Glo™ Lysis Reagent to the deposit sample, and then secure the cap onto the tube.
4. Mix by inverting the tube at least five times to resuspend the deposit in the Water-Glo™ Lysis Reagent. Allow the Lysis Reagent to fully extract the cellular content of the deposit by allowing it to act for 5 minutes.
5. Remove 100µl of the liquid from the sample and place it in a clean 1.5ml microfuge tube, then add 100µl of Water-Glo™ Detection Reagent. Immediately read the luminescence signal using the GloMax® 20/20 Luminometer (RLU_{sample}).
6. Negative Control: Add 100µl of Lysis Reagent to a 1.5ml microfuge tube, then add 100µl of Water-Glo™ Detection Reagent. Immediately read the luminescence using the GloMax® 20/20 Luminometer ($RLU_{lysis\ reagent}$).
7. Positive Control: Add 100µl of the ATP Standard to a 1.5ml microfuge tube, then add 100µl of Water-Glo™ Detection Reagent. Immediately read the luminescence signal using the GloMax® 20/20 Luminometer ($RLU_{ATP\ standard}$).
8. Calculate the Total ATP of the sample using the formula below:

$$\text{Total ATP } (pg/g) = \frac{RLU_{sample} - RLU_{lysis\ reagent}}{RLU_{ATP\ standard} - RLU_{lysis\ reagent}} \times \frac{5{,}000pg}{\text{Weight } (g)}$$

Example: Weight = 1 gram (g)

Measurement of Total Biocontamination from Biofilm Collection Coupons (e.g., corrosion coupons, biofilm formation)

1. Record the time (in days) that the coupon was placed in the water system for biofilm formation or corrosion study. This is the incubation time (t). For calculation of biofilm formation rate (BFR), record the incubation time (t) and the surface area of the coupon (cm^2).
2. Reconstitute the Water-Glo™ Detection Reagent by adding the Water-Glo™ Buffer to the lyophilized Water-Glo™ Substrate.
3. Take the biofilm collection device (e.g., corrosion coupon, biofilm formation coupon) and place it into a 15ml tube containing 5ml of Water-Glo™ Lysis Reagent. The entire coupon should come in contact with the lysis reagent. If the coupon does not fit in the 15ml tube, obtain a larger container.
4. Gently shake the tube containing the coupon on an orbital shaker, or invert the tube at least five times. Ensure coupon is completely immersed in Lysis Reagent for at least 5 minutes (including shake time).
5. Remove 100µl of the liquid from the sample tube (this will contain extracted sample) and place it in a clean 1.5ml microfuge tube, then add 100µl of Water-Glo™ Detection Reagent. Immediately read the luminescence signal using the GloMax® 20/20 Luminometer (RLU_{sample}).
6. Measurement of the blank: Add 100µl of Lysis Reagent to a 1.5ml microfuge tube, then add 100µl of Water-Glo™ Detection Reagent. Immediately read the luminescence signal using the GloMax® 20/20 Luminometer ($RLU_{lysis\ reagent}$).
7. Measurement of ATP standard: Add 100µl of the Total ATP Standard to a 1.5ml microfuge tube, then add 100µl of Water-Glo™ Detection Reagent. Immediately read the luminescence signal using the GloMax® 20/20 Luminometer ($RLU_{ATP\ standard}$).
8. Calculate the Total ATP (picogram/device) using the formula below:

$$\text{Total ATP (pg/device)} = \frac{RLU_{sample} - RLU_{lysis\ reagent}}{RLU_{ATP\ standard} - RLU_{lysis\ reagent}} \times \frac{5{,}000pg}{1\ device}$$

Alternatively, you may calculate Total ATP (picogram/cm^2) using the formula below if the suface area of the coupon is known.

$$\text{Total ATP (pg/cm}^2\text{) (coupons)} = \frac{RLU_{sample} - RLU_{lysis\ reagent}}{RLU_{ATP\ standard} - RLU_{lysis\ reagent}} \times \frac{5{,}000pg}{\text{Surface Area (cm}^2\text{)}}$$

To calculate biofilm formation rate (BFR; pg ATP/cm^2/days), use the modified formula below:

$$\text{Biofilm Formation Rate (BFR) (pg ATP/cm}^2\text{/days)} = \frac{RLU_{sample} - RLU_{lysis\ reagent}}{RLU_{ATP\ standard} - RLU_{lysis\ reagent}} \times \frac{5{,}000pg}{\text{Surface Area (cm}^2\text{)} \times \text{Incubation Time (days)}}$$