

TECHNICAL MANUAL

Water-Glo™ System

Instructions for Use of Products

AM1001, AM1002, AM1003, AM1004 and AM1005



Water-Glo™ System

All technical literature is available at: www.promega.com/protocols/
 Visit the web site to verify that you are using the most current version of this Technical Manual.
 E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

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1. Description

Microbial growth in industrial water systems is a serious problem that can cause biofilm formation (biofouling) and microbiologically induced corrosion (MIC), leading to poor water quality and costly mitigation. To prevent microbial growth, it is important to regularly monitor the amount of biomass in water. This can be done by measuring ATP (adenosine triphosphate) concentrations in water samples. The Water-Glo™ System is designed to measure ATP from viable cells in complex aqueous samples, such as drinking water, sea water, well water, waste water, water in reservoirs and other industrial water systems. The system uses a luciferase enzyme that generates luminescence upon reacting with ATP. The luminescent signal directly correlates with the level of ATP as well as the amount of active biomass in the water sample.

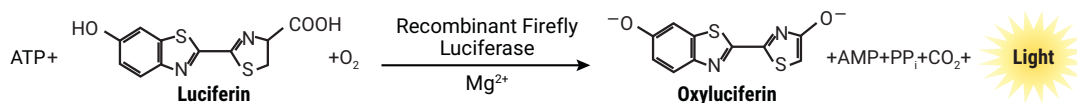


Figure 1. Schematic diagram of Water-Glo™ technology.

Complex water sample matrices and operational limitations at facilities have restricted the wide adoption of ATP for measurement of active biomass. To address these issues, the Water-Glo™ System incorporates an easy-to-use workflow to allow flexibility in sample preparation. The reagents are extremely sensitive and resistant to environmental factors, such as storage temperature and chemical inhibitors.

In the Water-Glo™ System^(a,b,c), filters are used to concentrate viable biomass from large volume samples by capturing that biomass on a filter surface. Small aqueous contaminants will pass through the filter, but biomass will not. For samples that contain oil and water mixtures, an additional organic wash step is used to remove inhibitory organic compounds from the filter. The captured biomass is extracted using the Water-Glo™ Lysis Reagent to release cellular ATP. The Water-Glo™ Lysis Reagent is designed to extract cellular components from hard-to-lyse samples such as biofilms. The extracted ATP is then detected using the Water-Glo™ Detection Reagent. The resulting luminescent signal is proportional to the amount of ATP present in the sample and can be measured using a luminometer.

The Water-Glo™ System complies with ASTM D4012 Standard Test Method for ATP Content of Microorganisms in Water. The Water-Glo™ System can also be used with additional sample types (e.g., swabs, solid samples, coupons, low bioburden samples and culture-based sterility testing). Contact us at applied@promega.com for additional protocols for these specific sample types, or visit www.promega.com

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Water-Glo™ Complete Aqueous	1 each	AM1001

Includes sufficient reagents and supplies for 20 tests:

- 1 vial Water-Glo™ Substrate (lyophilized)
- 1 × 10ml Water-Glo™ Buffer
- 2 × 65ml Water-Glo™ Lysis Reagent
- 1 × 2ml Water-Glo™ ATP Standard (1ng/ml or 1,000pg/ml)
- 20 each 20ml Syringe
- 20 each 0.2µm Syringe Filter
- 20 each 1.5ml Tube
- 20 each 15ml Tube

PRODUCT	SIZE	CAT.#
Water-Glo™ Reagents Aqueous	1 each	AM1002

Includes sufficient reagents for 20+ tests:

- 1 vial Water-Glo™ Substrate
- 1 × 10ml Water-Glo™ Buffer
- 2 × 65ml Water-Glo™ Lysis Reagent
- 1 × 2ml Water-Glo™ ATP Standard (1ng/ml or 1,000pg/ml)

PRODUCT	SIZE	CAT.#
Water-Glo™ 96 Reagents Aqueous	1 each	AM1003

Includes sufficient reagents for 400 tests using 96-well filter plates:

- 1 vial Water-Glo™ Substrate
- 1 × 60ml Water-Glo™ Buffer
- 1 × 65ml Water-Glo™ Lysis Reagent
- 1 × 2ml Water-Glo™ ATP Standard (1ng/ml or 1,000pg/ml)

PRODUCT	SIZE	CAT.#
Water-Glo™ Reagents Organic	1 each	AM1004

Includes sufficient reagents for 20 tests:

- 1 vial Water-Glo™ Substrate
- 1 × 10ml Water-Glo™ Buffer
- 2 × 65ml Water-Glo™ Lysis Reagent
- 1 × 2ml Water-Glo™ ATP Standard (1ng/ml or 1,000pg/ml)
- 1 × 50ml Water-Glo™ Organic Wash Solution (available separately, Cat.# AM1041)



2. Product Components and Storage Conditions (continued)

PRODUCT	SIZE	CAT.#
Water-Glo™ 96 Reagents Organic	1 each	AM1005

Includes sufficient reagents for 400 tests using 96-well filter plates:

- 1 vial Water-Glo™ Substrate
- 1 × 60ml Water-Glo™ Buffer
- 1 × 65ml Water-Glo™ Lysis Reagent
- 1 × 2ml Water-Glo™ ATP Standard (1ng/ml or 1,000pg/ml)
- 1 × 50ml Water-Glo™ Organic Wash Solution (available separately, Cat.# AM1041)

Storage Conditions: All components can be stored at room temperature (15–30 °C) prior to initial use. The reconstituted Water-Glo™ Detection Reagent can be stored in a refrigerator (4°C) for 6 months or at room temperature for two weeks without significant loss of performance. All other components can be stored at room temperature after initial use.

Waste Disposal: Follow local and federal regulatory guidelines for waste disposal. Typically, reagents can be disposed down the drain if flushed with plenty of water.

3. Single Test Protocol

Note: Use this protocol for Water-Glo™ Complete Aqueous (Cat.# AM1001) and Water-Glo™ Reagents Aqueous (Cat.# AM1002). If your sample contains oil, please use the protocol described in Section 5.

3.A. Materials to be Supplied by the User

- tube luminometer (e.g., GloMax® 20/20 Luminometer, Cat. # E5311)
- syringe filter (required for Cat.# AM1002; e.g., Sartorius Minisart® GF Syringe Filters, Order# 17824K)
- micropipette
- waste container

3.B. Protocol for Sample Processing

! It is very important to use clean equipment and wear new, disposable gloves to prevent contamination by trace amounts of ATP present in fingerprints, glassware, etc. Do not touch the outside of the gloves with your fingers or skin.

1. Shake the water sample container up and down several times to ensure complete mixing.
Note: If the water sample is stored in a refrigerator (4°C), let it sit at room temperature for at least 30 minutes before mixing.
2. Draw water sample into the Syringe by pulling on the plunger. Attach the Syringe Filter to the Syringe. Set the Syringe over a waste container and push the plunger to discard filtered water sample into the waste container. Biomass in the water sample will be captured on the Syringe Filter.
3. If you need to process more volume of the same water sample, detach the Syringe Filter and repeat Step 2 using the same Syringe Filter.

! Always remove the Syringe Filter before pulling back the plunger.

4. Record the total volume (ml) of sample used (V_{sample}) into **Box A** of the ATP Calculation Worksheet (see Section 6).
5. After the water sample has passed through the Syringe, detach the Syringe Filter and pull the plunger out. Re-attach the Syringe Filter to the Syringe. Use a pipette to transfer 2ml or 5ml of Water-Glo™ Lysis Reagent into the Syringe according to the water sample condition (see table below).

Water Sample Condition	Estimated Biomass	Volume of Water-Glo™ Lysis Reagent
Clear	Low	2ml
Turbid	High	5ml

Note: Using too little Water-Glo™ Lysis Reagent may underestimate the amount of ATP in the sample.

6. Record the volume (ml) of Water-Glo™ Lysis Reagent used ($V_{\text{lysis reagent}}$) in **Box B** of the ATP Calculation Worksheet (Section 6).
7. **Optional:** If an extended lysis time is desired (e.g., water sample contains biofilm), push the plunger just enough to wet the Syringe Filter, and let it sit at room temperature for 10 minutes.
8. Set the Syringe over a clean 15ml Collection Tube and push the plunger, collecting lysate flowthrough in the Collection Tube.
Note: The Water-Glo™ Lysis Reagent will extract ATP from the biomass and protect the ATP. ATP in the lysate is stable at room temperature for at least 24 hours.
9. If you have multiple water samples, process all samples up to this step. Proceed to Section 3.C to perform ATP detection on each sample.

3.C. Protocol for ATP Detection

1. Prepare the Water-Glo™ Detection Reagent: Add the whole bottle of Water-Glo™ Buffer into the brown-colored vial containing lyophilized Water-Glo™ Substrate. Swirl the vial several times to mix.

Note: The Water-Glo™ Detection Reagent can be stored in a refrigerator (4°C) for 6 months or at room temperature for two weeks without significant loss of performance. If you are using Water-Glo™ Detection Reagent stored at 4°C, let it sit at room temperature for 30 minutes before use.

2. Use a micropipette to add 100µl of sample lysate and 100µl of Water-Glo™ Detection Reagent into a clean 1.5ml microfuge tube. Close the lid and swirl to mix. Then immediately place the microfuge tube in a tube luminometer and measure luminescent signal. We recommend setting the integration time to 1 second.

Note: To ensure consistent results, the time delay between mixing and measurement should be the same for every sample.

3. Record the RLU value (RLU_{sample}) into **Box C** of the ATP Calculation Worksheet (Section 6).

4. **Positive Control:** Add 100µl of the Water-Glo™ ATP Standard (1,000pg/ml) and 100µl of Water-Glo™ Detection Reagent into a clean 1.5ml microfuge tube. Measure luminescent signal immediately using a luminometer.

Note: Do not dilute the Water-Glo™ ATP Standard in water. If a lower concentration is needed, dilute in the Water-Glo™ Lysis Reagent.

5. Record the RLU value ($RLU_{\text{ATP standard}}$) into **Box D** of the ATP Calculation Worksheet (Section 6).

6. **Negative Control:** Add 100µl of Water-Glo™ Lysis Reagent and 100µl of Water-Glo™ Detection Reagent into a clean 1.5ml microfuge tube. Measure luminescent signal immediately using a luminometer.

Note: The Water-Glo™ Detection Reagent can be stored in a refrigerator (4°C) for 6 months or at room temperature for two weeks without significant loss of performance. All other reagents can be stored at room temperature after initial use.

7. Record the RLU value ($RLU_{\text{lysis reagent}}$) into **Box E** of the ATP Calculation Worksheet (Section 6).

8. Calculate the ATP concentrations using the ATP Calculation Worksheet (Section 6). Alternatively, use the Water-Glo™ ATP Calculator Tool available online at:

www.promega.com/resources/tools/water-glo-atp-calculator-tool

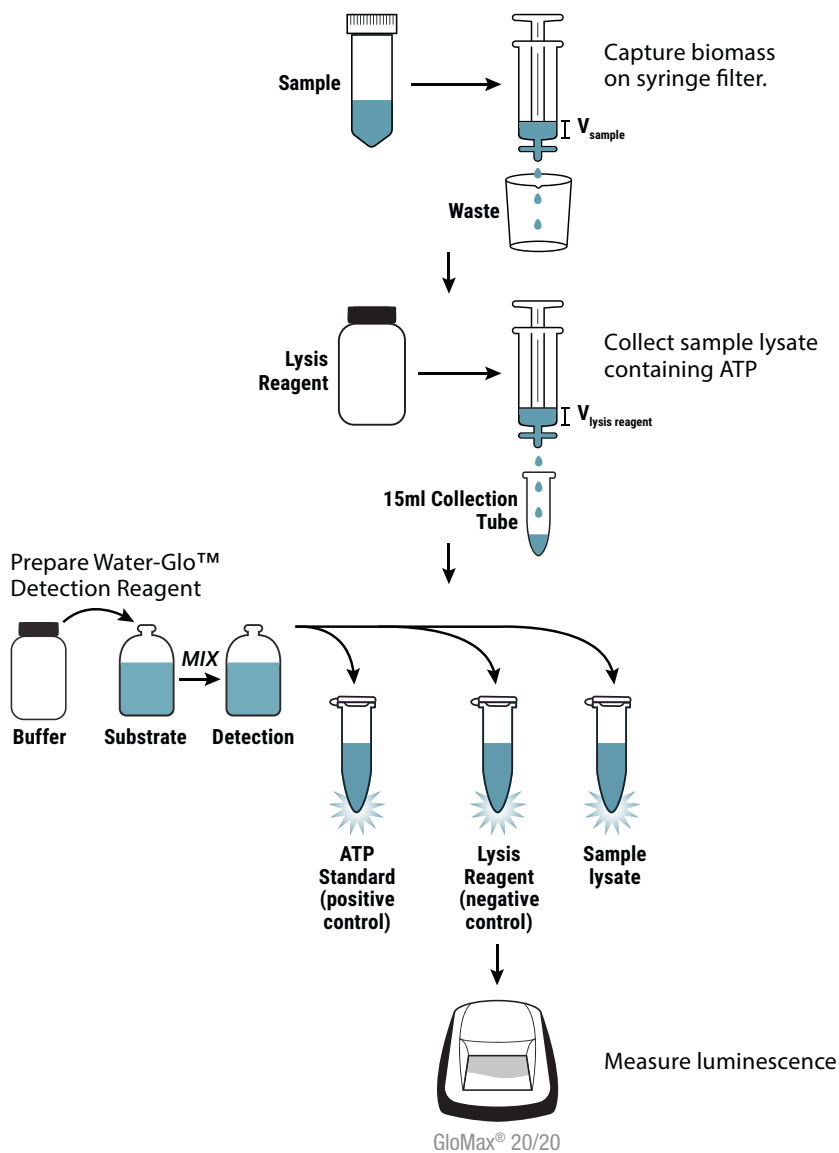


Figure 2. Water-Glo™ single test protocol.

4. 96-Well Batch Protocol

This section describes a batch method for processing larger numbers of samples (Cat.# AM1003 and AM1005). This method saves time and reagents by using 96-well filter plates and a plate-reading luminometer to process multiple samples at a time. **Note:** The batch method allows a maximum sample volume of 2ml/well to be filtered at a time. Sample volumes exceeding 2ml cannot be processed using the batch method.

4.A. Materials to be Supplied by the User

- 96-well filter plate (e.g., 96-Well Filter Plate 2ml 0.2µm wwPTFE, Promega Cat.# AM8782; AcroPrep™ Advance 96-Well Filter Plate, 2ml, 0.2µm or 0.45µm GHP membrane, Pall Cat.# 8782, 8784, MultiScreen® 96 Well High Volume Filter Plate, 0.45µm, Millipore Cat.# MVHVN4525) **Note:** If your sample contains oil, use a filter plate that is compatible with the sample matrix (e.g., AcroPrep™ Advance 96-Well Fiber Plate, 2ml, 1.0 µm Glass Fiber, Pall Cat.# 8231)
- solid flat-bottom white 96-well plate (e.g., Corning Cat.# 3912)
- robotic reservoir (e.g., Vacuum Manifold Collection Tray, Cat.# AM1300)
- vacuum manifold for 96-well plate (e.g., Vacuum Manifold for Water-Glo™ 96, Cat.# AM5017)
- Welch® Vacuum Pump (Model 2522B-01 for North America electrical, Cat.# A6720; Model 2522C-02 for Europe electrical, Cat.# A6722; Model 2522C-05 for Japan electrical, Cat.# A6724)
- GloMax® Navigator with Dual Injector (Cat.# GM2010), GloMax® Explorer (Cat.# GM3500), or GloMax® Discover System (Cat.# GM3000)
- ATP-free water (e.g., ATP-Free Water, Cat.# AM1101)
- 70% ethanol (e.g., 70% Ethanol Solution, Cat.# AM1091)
- 1M HCl, 1M NaOH (Optional for cleaning injector tubing)


4.B. Protocol for Sample Processing

 It is very important to use clean equipment and wear new, disposable gloves to prevent contamination by trace amounts of ATP present in fingerprints, glassware, etc. Do not touch the outside of the gloves with your fingers or skin.

1. Assemble the vacuum manifold according to the manufacturer's instructions. Place a 96-well filter plate on a waste reservoir.
2. Shake the water sample container up and down several times to ensure complete mixing. Add a maximum of 2ml of water sample into each well of the filter plate.

Note: The same water sample should be tested in duplicates or triplicates. Leave 4–8 wells empty for addition of positive and negative controls (Steps 8 and 9). See Figure 3 for example plate layout.

3. Record the volume (ml) of water sample used (V_{sample}) into **Box A** of the ATP Calculation Worksheet (see Section 6).
4. Apply vacuum (15–20inHg, 40–50cmHg, 0.5 bar or 10psi) until the entire water sample has passed through the filter. Biomass in the water sample will be captured on the filter plate.

 **Note:** It may take longer to vacuum if there are empty wells in the filter plate. To increase vacuum speed, use tape to cover the top of empty wells.

5. **Optional:** If your sample contains oil, add 125µl Water-Glo™ Organic Wash Solution to each well and apply vacuum until all liquid has passed.
6. Remove the waste reservoir, and place the filter plate on a clean white 96-well plate. Assemble the vacuum manifold according to the manufacturer's instructions.
7. Add 125µl (0.125ml) of Water-Glo™ Lysis Reagent to each well of the filter plate. Record this volume (ml; $V_{\text{lysis reagent}}$) in **Box B** of the ATP Calculation Worksheet (Section 6).
8. Apply vacuum until all the liquid has passed through the filter and collected in the white 96-well plate.
9. **Positive Control:** Add 125µl of Water-Glo™ ATP Standard into 2–4 empty wells of the white 96-well plate (see Figure 3 for example plate layout).
Note: Do not dilute the Water-Glo™ ATP Standard in water. If a lower concentration is needed, dilute in the Water-Glo™ Lysis Reagent.
10. **Negative Control:** Add 125µl of Water-Glo™ Lysis Reagent into 2–4 empty wells of the white 96-well plate (see Figure 3 for example plate layout).
11. Proceed to Section 4.C for instructions on ATP detection using the GloMax® Instrument.

Example Plate Layout												
	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Lysis Reagent
B	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Lysis Reagent
C	Sample 12	Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Lysis Reagent
D	Sample 12	Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Lysis Reagent
E	Sample 23	Sample 24	Sample 25	Sample 26	Sample 27	Sample 28	Sample 29	Sample 30	Sample 31	Sample 32	Sample 33	ATP Standard
F	Sample 23	Sample 24	Sample 25	Sample 26	Sample 27	Sample 28	Sample 29	Sample 30	Sample 31	Sample 32	Sample 33	ATP Standard
G	Sample 34	Sample 35	Sample 36	Sample 37	Sample 38	Sample 39	Sample 40	Sample 41	Sample 42	Sample 43	Sample 44	ATP Standard
H	Sample 34	Sample 35	Sample 36	Sample 37	Sample 38	Sample 39	Sample 40	Sample 41	Sample 42	Sample 43	Sample 44	ATP Standard

Figure 3. Example plate layout for Water-Glo™ 96-well batch method.

Note: For highly-variable samples, use triplicates for more representative sampling.

4.C. Protocol for ATP Detection using the GloMax® Instrument

1. Prepare the Water-Glo™ Detection Reagent: Add the whole bottle of Water-Glo™ Buffer into the brown-colored vial containing lyophilized Water-Glo™ Substrate. Swirl the vial several times to mix.
Note: The Water-Glo™ Detection Reagent can be stored in a refrigerator (4°C) for 6 months or at room temperature for two weeks without significant loss of performance. If you are using Water-Glo™ Detection Reagent stored at 4°C, let it sit at room temperature for 30 minutes before use.
2. Prepare GloMax® Instrument injector: Flush injector five times with ATP-free water, followed by five times with 70% ethanol and another five times with ATP-free water. Prime the injector with 1ml of Water-Glo™ Detection Reagent.
3. Place the white 96-well plate containing the lysate inside the GloMax® Instrument. Start the Water-Glo™ 96 protocol (Kinetics mode; Inject: 125µl, Speed: 200µl/second, Wait: 2 sec; Luminescence: 2 second integration time, Reading: 1, Interval: 2 seconds).
4. Clean the GloMax® Instrument injector: Flush injector five times with ATP-free water, followed by five times with 70% ethanol, another five times with ATP-free water and then five times with air.
Note: To prevent biofilm buildup in the injector which may result in high background, we recommend occasional cleaning with acid and base solutions. See Section 4.D. for detailed instructions.
5. Export data from the GloMax® Instrument. To calculate ATP concentrations, copy and paste RLU values into the Water-Glo™ ATP Calculator Tool available online at:
www.promega.com/resources/tools/water-glo-atp-calculator-tool

4.D. Protocol for Cleaning the Injector

Biofilm buildup in the GloMax® Instrument injector can result in high background signal (>1,000 RLU for Water-Glo™ Lysis Reagent only). This section describes a cleaning cycle with acid and base solutions to remove and prevent biofilm buildup. **Note:** The Luminometer Injector Tubing Cleaning Kit (available separately; Cat.# AM1110) contains 70% ethanol and ATP-free water for injector cleaning.

1. (Optional) Flush the injector five times with 1M HCl, then five times with ATP-free water.
2. Flush the injector five times with 1M NaOH.
Note: Alternatively, you may use 10% bleach solution instead of acid and base solutions.
3. Flush the injector five times with ATP-free water.
4. Confirm sufficient cleaning: Add Water-Glo™ Lysis Reagent into at least five wells of a new, clean white 96-well plate. Place the plate in the GloMax® instrument and run the Water-Glo™ 96 Protocol. An average signal under 1,000 RLU suggests the cleaning was sufficient to remove biofilm. If the average signal is over 1,000 RLU, repeat the cleaning cycle including the optional 1M HCl wash (Step 1) or replace the injector tubing (See Section 9 for replacement tubing ordering information).
Note: For instructions on how to replace the injector tubing, please refer to the appropriate GloMax® Instrument operating manual available at **www.promega.com/protocols**

Instructional videos for replacing the injector tubing on GloMax® Instruments are available at **www.promega.com/resources/multimedia**

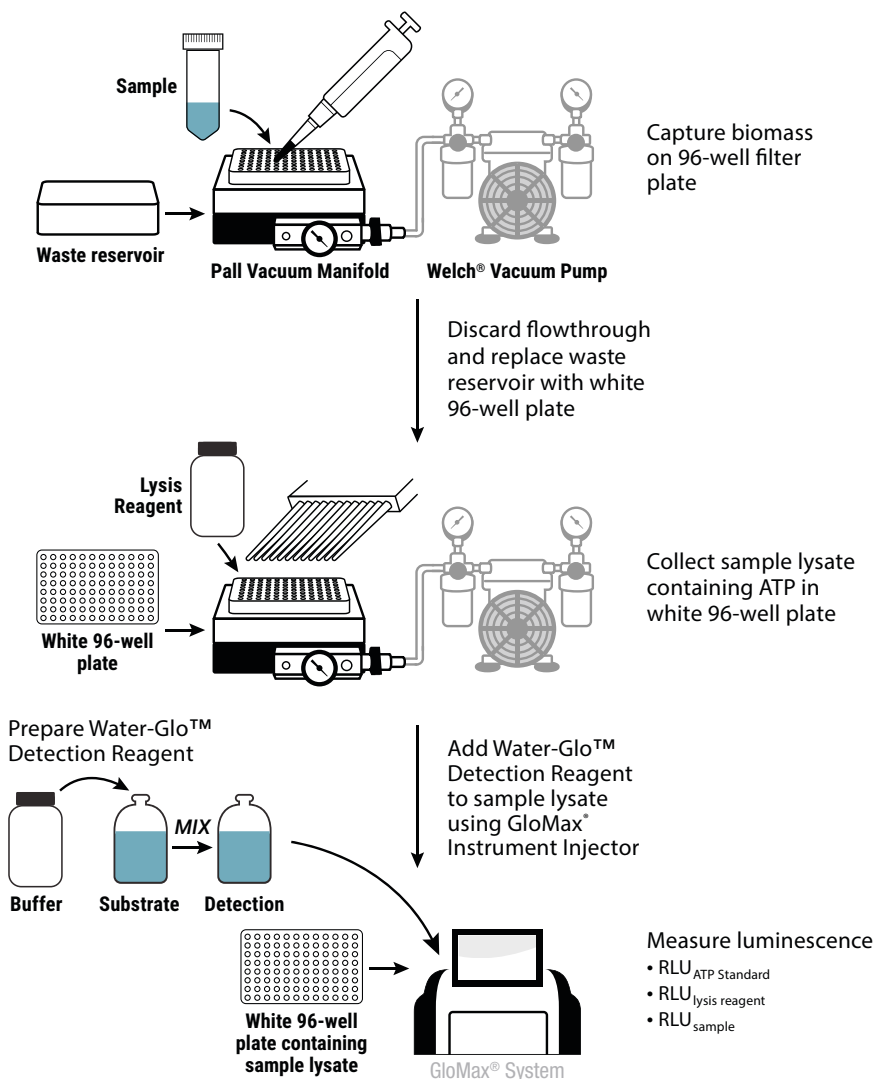


Figure 4. Water-Glo™ 96-well batch protocol.

5. Single Test Protocol for Samples Containing Oil

This section describes the protocol for samples that contain a mixture of oil and water (Cat.# AM1004). These samples may require an additional wash step of the Syringe Filter with the Water-Glo™ Organic Wash Solution (Cat.# AM1041) prior to cell lysis and extraction of cellular ATP.


5.A. Materials to be Supplied by the User

- Water-Glo™ Organic Wash Solution (Cat.# AM1041)
- tube luminometer (e.g., GloMax® 20/20 Luminometer, Cat.# E5311)
- syringe filter (e.g., Sartorius Minisart® GF Syringe Filters, Order# 17824K)
- micropipette
- waste container

5.B. Protocol for Sample Processing



It is very important to use clean equipment and wear new, disposable gloves to prevent contamination by trace amounts of ATP present in fingerprints, glassware, etc. Do not touch the outside of the gloves with your fingers or skin.

1. Shake the water sample container up and down several times to ensure complete mixing.
Note: If the water sample is stored in a refrigerator (4°C), let it sit at room temperature for at least 30 minutes before mixing.
 2. Draw water sample into the Syringe by pulling on the plunger. Attach the Syringe Filter to the Syringe. Set the Syringe over a waste container and push the plunger to discard filtered water sample into the waste container. Biomass in the water sample will be captured on the Syringe Filter.
 3. If you need to process more volume of the same water sample, detach the Syringe Filter and repeat Step 2 using the same Syringe Filter.
- 
- Always remove the Syringe Filter before pulling back the plunger.
4. Record the total volume (ml) of sample used (V_{sample}) into **Box A** of the ATP Calculation Worksheet (see Section 6).
 5. Detach the Syringe Filter, and draw the Water-Glo™ Organic Wash Solution into the syringe. Then attach the filter and push the plunger to pass the Organic Wash Solution into a waste container. This step removes organic contaminants from the water sample.
 6. After the water sample has passed through the Syringe, detach the Syringe Filter and pull the plunger out. Re-attach the Syringe Filter to the Syringe. Use a pipette to transfer 2ml or 5ml of Water-Glo™ Lysis Reagent into the Syringe according to the water sample condition (see table below).

Water Sample Condition	Estimated Biomass	Volume of Water-Glo™ Lysis Reagent
Clear	Low	2ml
Turbid	High	5ml

Note: Using too little Water-Glo™ Lysis Reagent may underestimate the amount of ATP in the sample.

7. Record the volume (ml) of Water-Glo™ Lysis Reagent used ($V_{\text{lysis reagent}}$) in **Box B** of the ATP Calculation Worksheet (Section 6).
8. **Optional:** If an extended lysis time is desired (e.g., water sample contains biofilm), push the plunger just enough to wet the Syringe Filter (allow a few drops to pass through), and let it sit at room temperature for 10 minutes.
9. Set the Syringe over a clean 15ml Collection Tube and push the plunger, collecting lysate flowthrough in the Collection Tube.
Note: The Water-Glo™ Lysis Reagent will extract ATP from the biomass and protect the ATP. The ATP in the lysate is stable at room temperature for at least 24 hours.
10. If you have multiple water samples, process all samples up to this step. Proceed to Section 5.C to perform ATP detection on each sample.

5.C. Protocol for ATP Detection

1. Prepare the Water-Glo™ Detection Reagent: Add the whole bottle of Water-Glo™ Buffer into the brown-colored vial containing lyophilized Water-Glo™ Substrate. Swirl the vial several times to mix.
Note: The Water-Glo™ Detection Reagent can be stored in a refrigerator (4°C) for 6 months or at room temperature for two weeks without significant loss of performance. If you are using Water-Glo™ Detection Reagent stored at 4°C, let it sit at room temperature for 30 minutes before use.
2. Use a micropipette to add 100µl of sample lysate and 100µl of Water-Glo™ Detection Reagent into a clean 1.5ml microfuge tube. Close the lid and swirl to mix. Then immediately place the microfuge tube in a tube luminometer and measure luminescent signal. We recommend setting the integration time to 1 second.
Note: To ensure consistent results, the time delay between mixing and measurement should be the same for every sample.
3. Record the RLU value (RLU_{sample}) into **Box C** of the ATP Calculation Worksheet (Section 6).
4. **Positive Control:** Add 100µl of the Water-Glo™ ATP Standard (1,000pg/ml) and 100µl of Water-Glo™ Detection Reagent into a clean 1.5ml microfuge tube. Measure luminescent signal immediately using a luminometer.
Note: Do not dilute the Water-Glo™ ATP Standard in water. If a lower concentration is needed, dilute in the Water-Glo™ Lysis Reagent.
5. Record the RLU value ($RLU_{\text{ATP standard}}$) into **Box D** of the ATP Calculation Worksheet (Section 6).
6. **Negative Control:** Add 100µl of Water-Glo™ Lysis Reagent and 100µl of Water-Glo™ Detection Reagent into a clean 1.5ml microfuge tube. Measure luminescent signal immediately using a luminometer.
Note: The Water-Glo™ Detection Reagent can be stored in a refrigerator (4°C) for 6 months or at room temperature for two weeks without significant loss of performance. All other reagents can be stored at room temperature after initial use.
7. Record the RLU value ($RLU_{\text{lysis reagent}}$) into **Box E** of the ATP Calculation Worksheet (Section 6).
8. Calculate the ATP concentrations using the ATP Calculation Worksheet (Section 6). Alternatively, use the Water-Glo™ ATP Calculator Tool available online at:
www.promega.com/resources/tools/water-glo-atp-calculator-tool

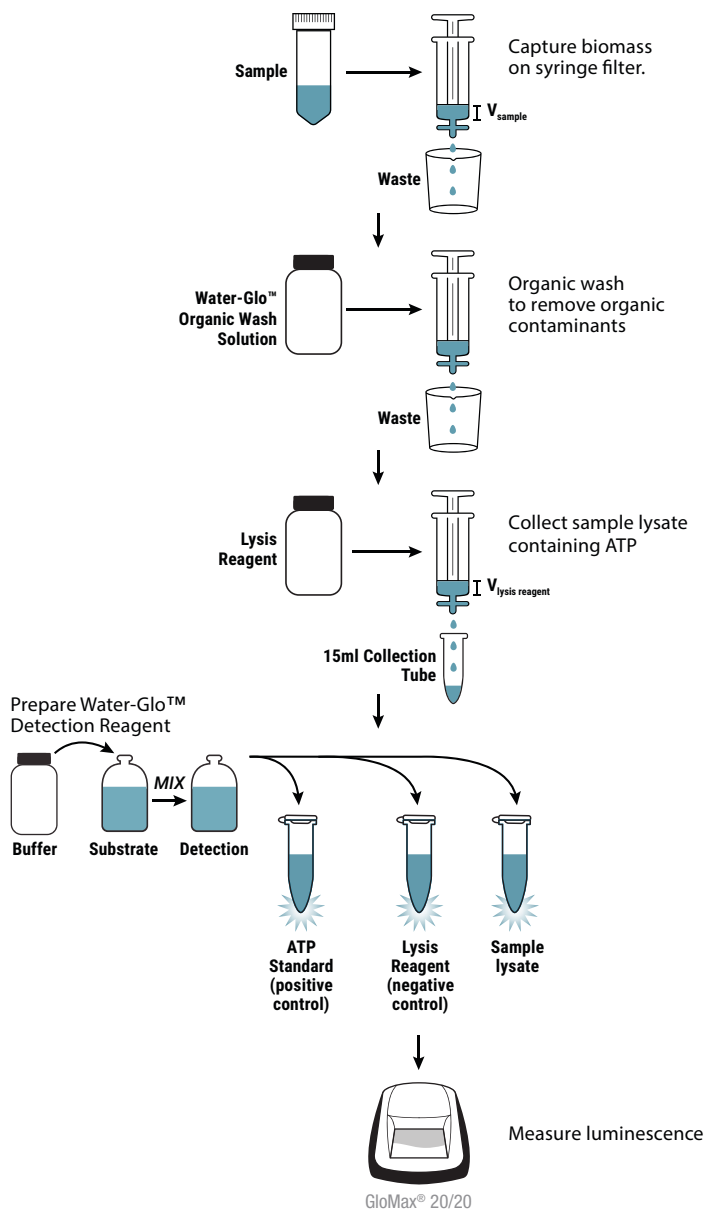


Figure 5. Water-Glo™ single test protocol for sample containing oil.

6. ATP Calculation Worksheet

This worksheet uses the following equation to calculate ATP concentration in water samples:

$$\text{ATP}(\text{pg/ml}) = \frac{\text{RLU}_{\text{sample}} - \text{RLU}_{\text{lysis reagent}}}{\text{RLU}_{\text{ATP standard}} - \text{RLU}_{\text{lysis reagent}}} \times \frac{V_{\text{lysis reagent}}}{V_{\text{sample}}} \times 1,000$$

Record volumes and RLU values in the boxes below:

Box A (V_{sample}): A ml

Box B ($V_{\text{lysis reagent}}$): B ml

Box C ($\text{RLU}_{\text{sample}}$): C

Box D ($\text{RLU}_{\text{ATP standard}}$): D

Box E ($\text{RLU}_{\text{lysis reagent}}$): E

Insert recorded numbers into the equation below to calculate ATP concentration. Alternatively, insert numbers into the Water-Glo™ ATP Calculator Tool available online at:

www.promega.com/resources/tools/water-glo-atp-calculator-tool

$$\text{ATP}(\text{pg/ml}) = \frac{\text{C } - \text{E } }{\text{D } - \text{E } } \times \frac{\text{B } }{\text{A } } \times 1,000$$

Note: If $\text{RLU}_{\text{sample}}$ is lower than $\text{RLU}_{\text{lysis reagent}}$, this suggests the ATP concentration in the water sample is close to 0. If $\text{RLU}_{\text{lysis reagent}}$ is severalfold higher than $\text{RLU}_{\text{sample}}$, there may be ATP contamination in the Water-Glo™ Lysis Reagent or the GloMax® Instrument injector tubing (see Troubleshooting, Section 8).

7. Guidelines for ATP Concentration in Water Samples

ATP concentration in water is extremely sensitive to changes in microbial quantity. Therefore, we recommend using ATP concentration as a monitoring tool to detect biomass and assess water quality. Monitor ATP concentration regularly to establish a baseline for your water system. High ATP concentration compared to the baseline could indicate a need for certain actions. Here, we provide suggested guidelines for ATP concentrations of various water sample types that may warrant additional monitoring or corrective action. However, we recommend establishing your own criteria for individual systems.

Water Type	Controlled Process (pg/ml ATP)	Additional Monitoring (pg/ml ATP)	Corrective Action (pg/ml ATP)	Input Volume/Test (ml)
Drinking water	<0.5	0.5 to 10	>10	2–20
Raw water (fresh, brackish or salt)	<10	10 to 100	>100	2–20
Cooling water (oxidizing biocide-treated)	<10	10 to 100	>100	2–20
Cooling water (non-oxidizing biocide-treated)	<100	100 to 1000	>1000	2–20
High-purity water	<0.1	0.1 to 1.0	>1.0	100

Note: The values in this table are general guidelines. Establish your own criteria for individual systems.

Correlation to Bacteria Number: One *E. coli* contains approximately 1 femtogram of ATP (1). Therefore, 1pg of ATP correlates to approximately 1,000 *E. coli* bacteria. However, we recommend you establish a more accurate correlation based on your individual system.

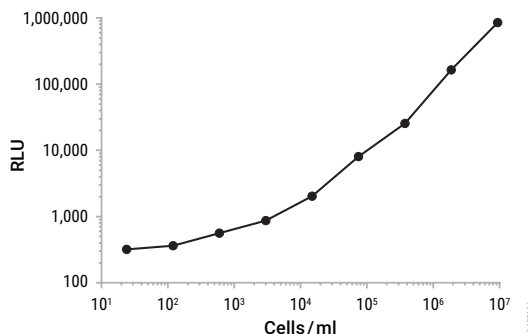


Figure 6. Titration of bacteria using Water-Glo™ reagents. 20ml of *E. coli* cells at various cell densities were filtered using a 0.2µm nylon filter. Captured biomass was extracted using 5ml of Water-Glo™ Lysis Reagent. Extracted ATP was measured by mixing 100µl of the lysate with 100µl of reconstituted Water-Glo™ Detection Reagent. Luminescence was recorded using the GloMax® 20/20. Experiment done in triplicates, results shown are mean ± standard deviation.

Limit of Detection: The following figures demonstrate the range of linear detection of ATP using Water-Glo™ reagents. We recommend you determine the limit of detection based on your individual system.

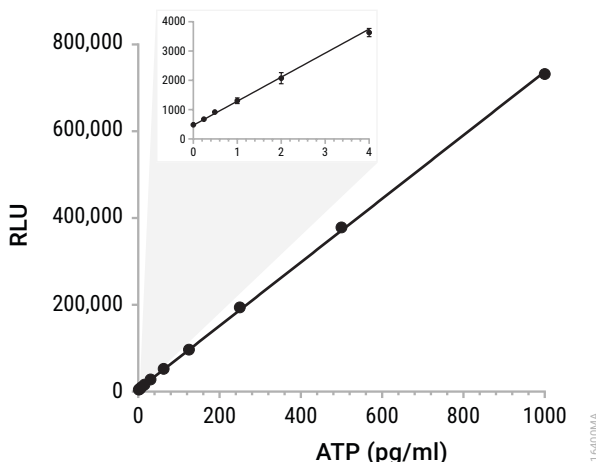


Figure 7. Titration of ATP using Water-Glo™ reagents measured on GloMax® 20/20. ATP was titrated in Water-Glo™ Lysis Reagent from 0–1,000pg/ml. 100µl of ATP sample was mixed with 100µl of reconstituted Water-Glo™ Detection Reagent and luminescence recorded using the GloMax® 20/20. Experiment was done in triplicates, results shown are mean ± standard deviation. Limit of detection of 0.03pg ATP (100µl of 0.3pg/ml) is achieved consistently.

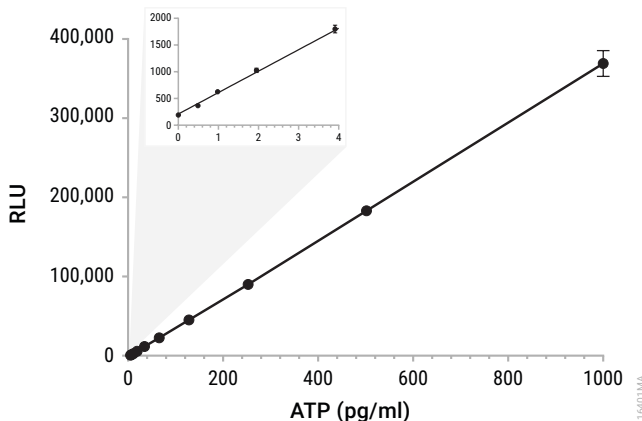


Figure 8. Titration of ATP using Water-Glo™ reagents measured on GloMax® Navigator. ATP was titrated in Water-Glo™ Lysis Reagent from 0–1,000pg/ml. 100µl of ATP sample was mixed with 100µl of reconstituted Water-Glo™ Detection Reagent and luminescence recorded using the GloMax® Navigator. Experiment was done in triplicates, results shown are mean ± standard deviation. Limit of detection of 0.05pg ATP (100µl of 0.5pg/ml) is achieved consistently.

8. References

- Hattori, N. *et al.* (2003) Enhanced microbial biomass assay using mutant luciferase resistant to benzalkonium chloride. *Anal. Biochem.* **319**, 287–95.

9. Troubleshooting

Symptoms	Causes and Comments
Excessive pressure when pushing water sample through the Syringe	There may be air bubbles in the Syringe between water sample and plunger. To remove air bubbles, detach Syringe Filter, flip Syringe so the opening is pointed up, allowing the air bubble to rise to the top. Push plunger just enough to remove the air.
High luminescent background from Water-Glo™ Lysis Reagent only	Water-Glo™ Lysis Reagent might be contaminated with ATP and should be discarded. To prevent contamination, <u>ensure proper pipetting practice. Do not touch pipette tips.</u> If the luminescent signal for the Water-Glo™ Lysis Reagent only (blank) exceeds 1,000 RLU, there may be biofilm buildup in the GloMax® Instrument injector. Clean the injector with acid and base solutions, or 10% bleach solution. Or replace the injector tubing (see Section 4.D. for detailed instructions).
High signal variability from samples with low levels of ATP or small expected changes in ATP	Doubling the amount of detection reagent may reduce variability in the output luminescent signal.

10. Related Products

Product	Size	Cat.#
BacTiter-Glo™ Microbial Cell Viability Assay	10ml	G8230
ENLITEN® ATP Assay System	100 assays	FF2000
GloMax® 20/20 Luminometer	1 each	E5311
GloMax® Navigator System with Dual Injectors	1 each	GM2010
Replacement Tubing Assembly for GloMax® Navigator (Set of 2)	1 each	GM2030
GloMax® Discover System	1 each	GM3000
GloMax® Discover Replacement Tubing Assembly*	1 set	GM3014
GloMax® Dual Injectors with Pumps	1 each	GM3030
GloMax® Explorer System	1 each	GM3500

10. Related Products (continued)

Product	Size	Cat.#
Nuclease-Free Water	500ml	P1197
Water-Glo™ Lysis Reagent	65ml	AM102A-C
Water-Glo™ ATP Standard	2ml	AM103A-C
70% Ethanol Solution	100ml	AM1091
ATP-Free Water	100ml	AM1101
Luminometer Injector Tubing Cleaning Kit	1 each	AM1110
Vacuum Manifold Collection Tray	4/pack	AM1300
Vacuum Manifold for Water-Glo™ 96	1 each	AM5017
96-Well Filter Plate 2ml 0.2µm wwPTFE	5/pack	AM8782

*This replacement tubing is also suitable for the GloMax® Explorer.

11. Summary of Changes

The following changes were made to the 12/19 revision of this document:

1. Updated catalogue numbers.
2. Added information regarding the limit of detection.

^(a)European Pat. No. 1131441 and Japanese Pat. No. 4520084.

^(b)U.S. Pat. Nos. 7,083,911, 7,452,663 and 7,732,128, European Pat. No. 1383914 and Japanese Pat. Nos. 4125600 and 4275715.

^(c)U.S. Pat. Nos. 7,741,067, 8,361,739 and 8,603,767, Japanese Pat. No. 4485470 and other patents pending.

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