AquaBluer™ Instruction Manual

General Information

Description

AquaBluer™ is a proprietary colorimetric and fluorescent redox indicator. Viable cells convert AquaBluer™ from its oxidized form (non-fluorescent, blue) to its reduced form (fluorescent, red). The fluorescence intensity of AquaBluer™ at 540 nm excitation / 590 nm emission (540ex/590em) is proportional to the number of viable cells in the sample. Therefore, AquaBluer™ can be used to assess cell viability, cell proliferation, and cytotoxicity. It is non-toxic, simple to use, sensitive, reproducible, and offers a broad assay range.

Specification

Product Name	AquaBluer™ Solution
Product #	AB6015M
Size	15 ml AquaBluer™ for 15,000 assays
Storage	Light sensitive, store tightly capped in the dark at 4-22 °C for 12 mons
MSDS	Available at www.boltii.com
Quality Control	Each lot has an A600/A570 ratio >1.3

Terms & Condition

Product Usage:
For *In Vitro* Laboratory Research Use Only. NOT to be administered to humans or used for medical diagnosis.

Warranties and Liabilities:
Boltii Diagnostics, Inc. accepts no responsibility and shall not be held liable for any loss, damage, expense, consequential, or accidental damage, including damage to property, person, or premises arising out of the use, the results of use, or the inability to use these products. Boltii Diagnostics, Inc. MAKES NO WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING, WITHOUT LIMITATION, WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE.

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AquaBluer™ is a trademark of MultiTarget Pharmaceuticals, LLC.

AquaBluer™ Cell Viability Assay Protocol

1. Set up your 96-well culture plates

Prepare a 6,000-8,000 cells/100 µL suspension. Set up the following wells in quadruplicate:

a. **No-cell control:** 100 µL medium only (for background subtraction)
b. **Vehicle control:** 100 µL cells with solvent of test compound (100% viability reference)
c. **Positive control** (optional): 100 µL cells with known cytotoxic compound
d. **Test compound:** 100 µL cells with 6–10 concentrations of test compound (1:1 serial dilutions around the estimated IC_{50})

Incubate the plate at 37 °C for 24–72 hours, inspecting cell death daily under a microscope. When ~90% cell death is observed at the highest drug concentration, proceed with the assay.

2. Perform the AquaBluer™ assay

a. Dilute 0.1 mL of AquaBluer™ to 10 mL of culture medium in a reagent reservoir.
b. Mix thoroughly by pipetting up and down 10 times.
c. Aspirate and remove the culture medium from each well, then add 100 µL of the diluted AquaBluer™ solution using a multi-channel pipette.
d. Incubate the plate in a CO_2 incubator at 37 °C for 4 hours.

3. Acquire raw data

After incubation, read the plate using a fluorescence plate reader at 540ex/590em (relative fluorescence units, RFU).

4. Calculate cell viability and IC_{50}

a. Subtract the average RFU of No-cell controls (background) from all other values.
b. Calculate % Viability using the formula:

$$\% \text{ Viability} = (RFU_{test} / RFU_{veh}) \times 100$$

where RFU_{veh} is the average RFU of the no-drug (vehicle) control.

c. Enter % Viability and corresponding log [test compound] values into GraphPad Prism or a free online IC_{50} calculator (e.g., ic50-calculator) and fit the data using a Four-Parameter Logistic Model to obtain IC_{50} values and dose–response curves.

Note: If a fluorescence plate reader is unavailable, absorbance readings can be used. Measure A_{570} and A_{600} for each well, subtract A_{600} from A_{570} to obtain ΔA (equivalent to "RFU"), and use ΔA values to calculate % viability and IC_{50} as described above.

AquaBluer™ Bacteria Viability Assay Protocol

1. Set up your 96-well culture plates

Prepare a 1,000 cells/100 µL suspension. Set up the following wells in quadruplicate:

a. **No-cell control:** 100 µL medium only (for background subtraction)
b. **Vehicle control:** 100 µL cells with solvent of test compound (100% viability reference)
c. **Positive control** (optional): 100 µL cells with a known antimicrobial agent
d. **Test compound:** 100 µL cells with 6–10 concentrations of test agent (1:1 serial dilutions around the estimated IC_{50})

Cover the plate with aluminum sheet and incubate in a 37 °C shaking incubator at 200-300 rpm (depending on your shaker) for 24 hours, proceed with the assay.

2. Perform the AquaBluer™ assay

a. Dilute 0.1 mL of 100x AquaBluer™ to 1 mL of culture medium in a 2 mL microfuge tube.
b. Vortex to mix well.
c. Add 10 µL of the diluted AquaBluer™ solution to each well with a repeater pipette.
d. Cover the plate and incubate in the 37 °C shaker at 200-300 rpm for 4 hours.

3. Acquire raw data

After incubation, read the plate using a fluorescence plate reader at 540ex/590em (relative fluorescence units, RFU).

4. Calculate cell viability and IC_{50}

a. Subtract the average RFU of No-cell controls (background) from all other values.
b. Calculate % Viability using the formula:

   $$\% \text{ Viability} = (RFU_{test} / RFU_{veh}) \times 100$$

   where RFU_{veh} is the average RFU of the no-drug (vehicle) control.

c. Enter % Viability and corresponding log [test compound] values into GraphPad Prism or a free online IC_{50} calculator (e.g., ic50-calculator) and fit the data using a Four-Parameter Logistic Model to obtain IC_{50} values and dose–response curves.

Note: If a fluorescence plate reader is unavailable, absorbance readings can be used. Measure A_{570} and A_{600} for each well, subtract A_{600} from A_{570} to obtain ΔA (equivalent to "RFU"), and use ΔA values to calculate % viability and IC_{50} as described above.

Frequently Asked Questions (FAQ)

1. Should I store AquaBluer™ in the freezer?

AquaBluer™ is stable for 12 months at 4–22 °C. For long-term storage, you may keep it at –20 °C, where its shelf life can be extended indefinitely. Always store it in complete darkness and minimize light exposure.

2. How many cells should I seed per well?

This depends on the cell type's growth rate and drug exposure duration. As a general guideline, seed 6,000–8,000 cells/well. After overnight incubation, the culture should be ~20–30% confluent, allowing for up to 72 hours of drug exposure before untreated control wells become overconfluent or apoptotic.

3. Can AquaBluer™-treated cultures be used for other assays?

Yes. AquaBluer™ is non-toxic, and treated cells can typically be used for subsequent analyses. Although it is unlikely to interfere with other bioassays, it is recommended to validate compatibility for your specific application. We routinely recover AquaBluer™-containing media for multiplex cytokine ELISA assays.

4. My plate reader does not have 540ex/590em filters. What should I do?

You can use any fluorescence filter set that covers 550 ± 20 nm (excitation) and 600 ± 20 nm (emission). For absorbance-based readings, use 570 ± 20 nm and 600 ± 20 nm filters.

5. My data does not fit the Four-Parameter Model. What should I do?

If your concentration range does not include data points near 100% and 0% viability, the Four-Parameter Model may not converge. To resolve this:

- Repeat the experiment with a broader concentration range (both higher and lower).
- If repeating is impractical, you may estimate missing values by assigning theoretical concentrations (e.g., –4 log for 95% viability and +4 log for 5% viability) to approximate the IC_{50}.