AquaBluer Instruction Manual

General Information

Description

AquaBluer™ is a proprietary colorimetric and fluorescent redox indicator. Viable cells turn AquaBluer™ from its oxidized form (nonfluorescent, blue) to the reduced form (fluorescent, red). The fluorescence intensity of AquaBluer™ at 540ex/590em is proportional to the number of viable cells in the sample. Therefore, it may be used to assess cell viability, cell proliferation, and cytotoxicity. AquaBluer™ is nontoxic, simple to use, sensitive, reproducible, and has a broad assay range.

Specification

Product Name	AquaBluer™ Cell Viability Assay Solution
Product #	6015
Size	15 ml AquaBluer™ solution for 15,000 assays
Contents	15 ml AquaBluer™ solution, Instruction Manual
MSDS	Available at www.aquaplasmid.com
Storage	Light sensitive, store tightly capped in the dark at 4-22 °C for 12 months
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.
Limited Product Warranty: We offer a LIMITED PRODUCT WARRANTY to our customers. This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by MultiTarget Pharmaceuticals. We shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.
Product Warning: May cause irritation. The toxicological properties of this material have not been fully investigated.

Patents, Trademarks & Copyrights

AquaBluer™ is a trademark of MultiTarget Pharmaceuticals, LLC. ©2017 Multitarget Pharmaceuticals, LLC. All rights reserved.

AquaBluer™ Cell Viability Assay Protocol

This protocol serves as an example to perform cytotoxicity assay using 1µl of AquaBluer™ for each well containing 100 µl of medium in a 96-well format (for 384-well and other formats, adjust the volumes accordingly).

1. Set up your 96-well culture plates

Seed the cells at 6000-8000 cells/100 µl/well in 96-well culture plates (you may use either opaque-walled or clear-walled plates) and let the cells grow overnight. Set up quadruplicate wells of 1) no-cell control (100 µl of medium, for background scattering subtraction), 2) vehicle control (100 µl of cells with the vehicle of test compound, as 100% viability), 3) positive control (optional, 100 µl of cells treated with a known cytotoxic compound), 4) test compound (100 µl of cells treated with 6-10 concentrations of 1:1 serially diluted test compound around its estimated IC50). Depending on the toxicity of the compound, incubate at 37 °C for 24-72 hours (inspect cell killing daily under the microscope, when ~90% cell death is observed at the higher drug concentration(s), it will be a good time to perform the AquaBluer™ assay).

2. Perform the AquaBluer™ assay

Add 0.1 ml of AquaBluer™ to 10 ml of culture medium in a reagent reservoir, and pipette up and down 10 times to mix well. Aspirate to remove the medium from the cell culture and add 100 µl of the diluted AquaBluer™ to each well with a multi-channel pipettor. Return the plate to the incubator and incubate for 4 hours.

3. Data acquisition and calculation

Remove the plates from the incubator. Place the plate in a fluorescence plate reader and read the fluorescence intensity at 540ex/590em. Data calculation may be done as follows: 1) Subtract the average of fluorescence values (RFU) of the no-cell controls (background) from all other RFU values. 2) Convert the test RFU values to % viability using the formula: % Viability = (RFUtest / RFUveh) x 100. 3) Enter the % viability values and corresponding log test compound concentrations into a non-linear regression program such as Prism and use the Four Parameter Model to obtain the IC50 values of your test compound and the dose-response curve.

(Note: If a fluorescence plate reader is unavailable, you may use an absorbance plate reader to acquire AquaBluer™ viability data by recording A570 and A600 for each well at the end of the AquaBluer™ incubation period, subtracting each A600 value from its corresponding A570 value, and then performing the three steps of data calculation above for fluorescence data to obtain the IC50.)

Frequently Asked Questions

Please read through these questions carefully. The answers provide additional helpful tips and useful information for the successful use of AquaBluer™.

1. Should I keep AquaBluer™ in the freezer?

AquaBluer™ solution is stable at 4-22 °C for 12 months. However, if stored at –20 °C, its shelf life could extend indefinitely. It is important to keep the solution in complete darkness and minimize its light exposure.

2. How many cells should I seed in each well?

It depends on how fast the cells propagate and how long you need to expose the cells to a test compound. In most cases, it's a good bet to seed 6000-8000 cells/well. After overnight incubation, you will likely have a 20-30% confluent culture to start drug exposure and have ~72 hours of drug exposure period before the no-drug control start to enter apoptosis due to overgrowth.

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

AquaBluer™ is nontoxic to the cells and you should be able to use those cells for subsequent cell analysis. It is unlikely to interfere with other bioassays, but you need to test it for your particular assays to be sure. We routinely recover the media containing AquaBluer™ for multiplex cytokine ELISA assays.

4. I don’t have the exact 540ex/590em fluorescence filter set, what can I do?

You may use any fluorescence filter set covering 550±20nm for excitation and 600±20nm for emission. Similarly, for absorbance reading, you can use a 570±20nm and 600±20nm filter set.

5. My data does not fit the Four Parameter Model, what can I do?

If you do not include a wide enough range of test concentrations in your experiment or your test compound is too potent or too weak, your experiment may not generate data points around 90-100% or 1-10% viability range. In these cases, the Four Parameter Model, which assumes a sigmoid dose-response curve, may not be able to find the IC50. In such cases, you can either repeat the experiment to include enough higher or lower test concentrations or use a theoretical -4 log concentration for 95% viability and/or a +4 log concentration for 5% viability to estimate the IC50 from your existing data points.