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™ Solution
Product #	6001, 6015
Size	6001: 1 ml AquaBluer for 1,000 assays 6015: 15 ml AquaBluer for 15,000 assays
MSDS	Available at www. multitargetpharm.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.

Warranties and Liabilities: MultiTarget Pharmaceuticals 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. MultiTarget Pharmaceuticals MAKES NO WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING, WITHOUT LIMITATION, WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE.

Patents, Trademarks & Copyrights

AquaBluer™ is a trademark of MultiTarget Pharmaceuticals, LLC.

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 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 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. Raw data acquisition

Remove the plate from the incubator. Place the plate in a fluorescence plate reader and read the fluorescence intensity (RFU) at 540ex/590em.

4. Viability and IC50 calculation

Subtract the average of RFU of the No-cell control (background) from all other RFU values. Convert the test RFU values to % viability using the formula: ***% Viability = (RFUtest / RFUveh) x 100***, where RFUveh is the average RFU of the No-drug wells. Enter the % viability values and corresponding log test compound concentrations into a non-linear regression program (GraphPad Prism) or free online IC50 calculator (ic50.tk) and use the Four Parameter Model to obtain the IC50 values and 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 use the difference (eq “RFU”) to calculate the % viability and then IC50 similarly.)

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 drug 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 drug concentrations, or if repeating the experiment is not practical you may 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.

MATERIAL SAFETY DATA SHEET (MSDS)

TRADE NAME: AquaBluer™ Solution
DATE OF ISSUE: March 1, 2015

SECTION I: PRODUCT AND MANUFACTURER INFORMATION
TRADE NAME: AquaBluer™ Solution

MultiTarget Pharmaceuticals, LLC
5050 Edison Ave Ste 214, Colorado Springs, CO 80915, USA
Telephone: 1-801-769-6586
DATE PREPARED: 03-01-2015

SECTION II: COMPOSITION / INFORMATION ON INGREDIENTS
SYNONYMS: AquaBluer™
CHEMICAL CHARACTERIZATION: Proprietary aqueous solution of non-hazardous chemicals.
HAZARDOUS COMPONENT: None.

SECTION III: HAZARD IDENTIFICATION
May be harmful if swallowed. May cause irritation. Avoid breathing vapors, or dusts. Avoid contact with eyes, skin, and clothes. Wash thoroughly after handling. Keep container closed.

SECTION IV: FIRST AID MEASURES
IF EYE CONTACT: Immediately flush eyes with copious amounts of water for at least 15 minutes. Assure adequate flushing of eyes by separating the eyelids with fingers.
IF SKIN CONTACT: Immediately wash skin with soap and copious amounts of water.
IF SWALLOWED: Wash out mouth with water provided person is conscious. Call a physician.

SECTION V: FIREFIGHTING MEASURES
EXTINGUISHING MEDIA: Use extinguishing media appropriate to surrounding fire conditions.
SPECIAL FIREFIGHTING PROCEDURES: Wear self-contained breathing apparatus and protective clothing to prevent contact with eyes and skin.

SECTION VI: ACCIDENTAL RELEASE MEASURES
PRECAUTIONARY MEASURES: Wear self-contained breathing apparatus, chemical safety goggles, rubber boots, and heavy rubber gloves.
CLEAN-UP PROCEDURES: Absorb on sand or vermiculite and place in closed container for disposal. Ventilate area and wash spill site after material pick-up is complete.

SECTION VII: HANDLING AND STORAGE
STORAGE: Store in the dark. Store tightly closed at 4-22 °C.

SECTION VIII: EXPOSURE CONTROLS AND PERSONAL PROTECTION
Chemical safety goggles. Rubber gloves. Safety shower and eye bath. Wash thoroughly after handling. Do not get in eyes, on skin, or on clothing.

SECTION IX: PHYSICAL AND CHEMICAL PROPERTIES
PHYSICAL STATE / FORM: Liquid
COLOR: Blue to pink
ODOR: N/A
pH: (20 °C) N/V
VISCOSITY: (20 °C) N/A
MELTING POINT: N/A

BOILING POINT: N/A
IGNITION TEMPERATURE: N/A
FLASHPOINT: N/A
EXPLOSION LEVEL: N/A
VAPOR PRESSURE: (20 °C) N/A
SPECIFIC GRAVITY: (20 °C) N/A
SOLUBILITY IN WATER: (20 °C) Soluble

SECTION X: STABILITY AND REACTIVITY
SUBSTANCES TO BE AVOIDED: N/A
HAZARDOUS, COMBUSTION, OR DECOMPOSITION PRODUCTS: N/A

SECTION XI: TOXICOLOGICAL INFORMATION
TOXICITY DATA: N/A
INHALATION: May be harmful by inhalation.
EYE CONTACT: May cause eye irritation.
SKIN CONTACT: May cause skin irritation.
INGESTION: May be harmful if swallowed.
PROLONGED EXPOSURE: N/A
CHRONIC EFFECTS: N/A
RTECS NUMBER: N/A
ADDITIONAL INFORMATION: THE CHEMICAL, PHYSICAL, AND TOXICOLOGICAL PROPERTIES HAVE NOT BEEN THOROUGHLY INVESTIGATED. Additional harmful properties cannot be ruled out. The product should be handled with the normal caution accorded chemicals.

SECTION XII: ECOLOGICAL INFORMATION
N/A

SECTION XIII: DISPOSAL CONSIDERATIONS
There are no uniform regulations for the disposal of chemicals or residues. Dispose of container and unused contents in accordance with federal, state and local requirements.

SECTION XIV: TRANSPORT INFORMATION
DOT: None of the components are regulated.

SECTION XV: REGULATORY INFORMATION
SARA: None of the components are regulated.

SECTION XVI: OTHER INFORMATION
DATE OF PREPARATION: March 1, 2015.
DISCLAIMER: For research use only. The above information is believe to be correct but does not purport to be all inclusive and should be used only as a guide. MultiTarget Pharmaceuticals shall not be held liable for any damages or other consequences resulting from handling or from contact with the above product.