

User's Manual and Instructions

QBlue Cell Viability Assay Kits (Z5030004, Z5030005) Non-radioactive Fluorescent Assay for Cell Proliferation and Cytotoxicity

DESCRIPTION

This homogeneous assay involves simply adding a single reagent, the QBlue Cell Viability reagent, to the cell culture and measuring the fluorescence intensity (excitation wavelength = 530 - 570 nm, emission wavelength = 590 - 620 nm) after an incubation step. The QBlue Cell Viability reagent, like other resazurin-based assays such as the Alamar Blue reagent, utilizes the redox dye resazurin which is not fluorescent, but upon reduction by metabolically active cells is converted into a highly fluorescent product (resorufin). Living cells can readily reduce this non-toxic reagent and the resulting increase in fluorescence intensity can be conveniently monitored using a fluorescence spectrophotometer or plate reader. Nonviable cells have no metabolic capacity and, thus, will not reduce the dye. Therefore, the fluorescence intensity observed in this assay is a true measure of the viable cells.

This reagent has been optimized for maximum sensitivity, reproducibility and long shelf-life. The homogeneous cell-based assay can be performed in multi-well plates. The reagent is compatible with all culture media and with all liquid handling systems for high-throughput screening applications in 96-well and 384-well plates. Applications include cell proliferation, cytotoxicity and apoptosis.

KEY FEATURES

Safe. Non-radioactive assay (cf. ³H-thymidine incorporation assay).

Sensitive and accurate. As low as 100 cells can be accurately quantified.

Time efficient. High-throughput assay in 96-well and 384-well plates allows simultaneous processing ten of thousands of samples per day.

Homogeneous and convenient. A single reagent and "mix-incubate-measure" type assay. No wash and reagent transfer steps are involved.

Robust and amenable to HTS: Z' factors of 0.6 to 0.9 are routinely observed in 96-well and 384-well plates. Can be readily automated on HTS liquid handling systems.

APPLICATIONS

Cell Proliferation: effects of cytokines, growth factor, nutrients.

Cytotoxicity and Apoptosis: evaluation of toxic compounds, anti-cancer antibodies, toxins, environmental pollutants etc.

Drug Discovery: high-throughput screening for anticancer drugs.

KIT CONTENTS

Catalog #	Size (assays)	Reagent
Z5030004	5,000	50 mL
Z5030005	10,000	100 mL

Control Reagent (Cat # Z5030008): 50 mg saponin (to be ordered separately).

Storage conditions. This reagent is light sensitive and should be stored in the provided amber bottle at 4°C. The Control Reagent is stable at -20°C. Shelf life: 12 months.

This Protocol can be downloaded from www.biochain.com.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

The QBlue Cell Viability assay is based on the conversion of the non-fluorescent reagent to fluorescent product by metabolically active cells. For most cells this reductive reaction takes 1 to 5 hours. The fluorescence intensity of the product is then quantified on a fluorescent microplate reader. Although most culture media contain phenol red, phenol red does not interfere with the assay. All data in Technical Notes were obtained in culture media containing phenol red.

Reagent Preparation:

Important: bring reagent to room temperature before use.

Procedure using 96-well plate:

1. Plate and culture cells (80 μ L) in black 96-well tissue culture plates. Typical culture medium contains DMEM, 10% fetal bovine serum and antibiotics (penicillin/ strepto-mycin, gentamycin, etc), amino acids and other nutrients. Assays can be performed on either adherent cells or cells in suspension. The number of cells can vary from 100 to 80,000 per well. The volume can vary from 50 to 150 μ L, although 80 μ L is used in this protocol. In addition to the test samples, control wells of culture medium containing no cells or cells treated with a toxic reagent such as 0.1% saponin should be included.

2. Add test compounds and controls and incubate cells for the desired period of time (typically overnight). It is recommended that assays be run in duplicate or triplicate. Compounds and controls (20 μ L) can be added in phosphate buffered saline (PBS) or culture medium. The Control reagent can be conveniently reconstituted with 5 mL PBS (1% saponin).

3. Equilibrate the QBlue Reagent to room temperature. Add 10 μ L (per 100 μ L of cell culture) of the reagent per well. The volume of the reagent can be adjusted depending on the volume of cell culture. Tap plate to mix cells with compounds. Incubate for 1 to 5 hours at 37°C.

4. Measure fluorescent intensity for each well on a fluorescence plate reader. If a Molecular Devices LJL Analyst is used, use the rhodamine filter sets (530nm excitation filter, 590nm emission filter and 570nm dichroic mirror).

Procedure using 384-well plate:

1. Plate and culture cells (40 μ L) in black 384-well tissue culture plates. The number of cells can vary from 100 to 20,000 per well. The volume can vary from 25 to 60 μ L, although 40 μ L is used in this protocol. In addition to the test samples, control wells of culture medium containing no cells or cells treated with a toxic reagent such as 0.1% saponin should be included.

2. Add test compounds and controls and incubate cells for the desired period of time. It is recommended that assays be run in duplicate or triplicate and that compounds be added in PBS or culture medium with a volume of 10 μ L.

3. Equilibrate QBlue Reagent to room temperature. Add 5 μ L Reagent (per 50 μ L of cell culture). Tap plate lightly to mix Reagent with cells. Incubate for 1 to 5 hours at 37°C.

4. Measure fluorescent intensity for each well on a fluorescence plate reader. If a Molecular Devices LJL Analyst is used, use the rhodamine filter sets (530nm excitation filter, 590nm emission filter and 570nm dichroic mirror).

GENERAL CONSIDERATIONS

Incubation time. The incubation time is dependent on the cell line. Some cell lines exhibit strong metabolic activity and, thus, require shorter incubation time than less metabolically active cell lines. The incubation time can be easily determined by reading the plate multiple times e.g. every 30 minutes after adding the QBlue reagent. In general, incubation for 1 to 5 hours is sufficient. Extensive incubation (such as >18 hours) may result in non-linear fluorescence response at high cell numbers.

Cell number. Generally the optimized QBlue reagent shows a broad range linear fluorescence response to the number of culturing cells. It is recommended to determine the number of cells per well that gives a highest signal:noise ratio. The optimal cell number can be easily determined by serial dilution of cells.

Controls. A positive control that is either cytotoxic or promotes cell proliferation can be run although it is not required. Saponin is a cytotoxic detergent that is available from BioChain (see Figure 2 in Technical Notes). A blank control, i.e., culture medium without cells or cells containing 0.1% saponin, should be done for each assay. The blank control determines background fluorescence that must be subtracted for data analysis.

DATA ANALYSIS

For cell proliferation or cytotoxicity assays, the activity of a test compound can be calculated as percent change in cell number as follows,

Activity (%) or Cell viability (%) = $100 \times (F_{\text{compd}} - F_0) / (F_{\text{ctrl}} - F_0)$ where F_{compd} and F_{ctrl} are the average fluorescence intensities in the presence and absence (vehicle control) of the test compound and F_0 the averaged blank control fluorescence intensity.

For dose-response studies, the data are plotted against compound concentration and the EC_{50} for proliferation assay and IC_{50} for cytotoxicity assay can be determined by non-linear regression analysis using Prism or other data analysis tools.

LITERATURE

Cell Proliferation assays

1. Kreisler M, Christoffers AB, Willershausen B, D'Hoedt B (2003). Low-level 809 nm GaAlAs laser irradiation increases the proliferation rate of human laryngeal carcinoma cells in vitro. *Lasers Med Sci.* 18(2):100-3.

2. Nordling MM, Glinghammar B, Karlsson PC, de Kok TM, Rafter JJ (2003). Effects on cell proliferation, activator protein-1 and genotoxicity by fecal water from patients with colorectal adenomas. *Scand J Gastroenterol.* 38(5):549-55.

3. Kipshidze N, Moussa I, Nikolaychik V, Chekanov V, Khanna A, Colombo A, Leon MB, Moses J (2002). Influence of Class I interferons on performance of vascular cells on stent material in vitro. *Cardiovasc Radiat Med.* 3(2):82-90.

4. Gloeckner H, Jonuleit T, Lemke HD (2001). Monitoring of cell viability and cell growth in a hollow-fiber bioreactor by use of the dye Alamar Blue. *J Immunol Methods.* 252(1-2):131-8.

Cytotoxicity Assays

5. Nociari MM, Shalev A, Benias P, Russo C (1998). A novel one-step, highly sensitive fluorometric assay to evaluate cell-mediated cytotoxicity. *J Immunol Methods.* 1998;213(2):157-67.

6. Mikus J, Steverding D (2000). A simple colorimetric method to screen drug cytotoxicity against Leishmania using the dye Alamar Blue. *Parasitol Int.* 48(3):265-9.

7. Byth HA, Mchunu BI, Dubery IA, Bornman L (2001). Assessment of a simple, non-toxic Alamar blue cell survival assay to monitor tomato cell viability. *Phytochem Anal.* 12(5):340-6.

8. Lee JK, Kim DB, Kim JI, Kim PY (2000). In vitro cytotoxicity tests on cultured human skin fibroblasts to predict skin irritation potential of surfactants. *Toxicol In Vitro.* 14(4):345-9.

Evaluation of cytotoxic effects of antibody

9. Gazzano-Santoro H, Ralph P, Ryskamp TC, Chen AB, Mukku VR (1997). A non-radioactive complement-dependent cytotoxicity assay for anti-CD20 monoclonal antibody. *J Immunol Methods.* 202(2):163-71.

in vitro chemosensitivity

10. Sawabe Y, Yamagishi H, Yamaguchi N, Yamamura Y, Oka T (1996). In vitro chemosensitivity of human pancreatic cancer cell lines. *Int J Pancreatol.* 20(3):185-90.

Screening of cytotoxic compounds

11. Mikus J, Steverding D (2000). A simple colorimetric method to screen drug cytotoxicity against Leishmania using the dye Alamar Blue. *Parasitol Int.* 48(3):265-9.

12. Breinholt V, Larsen JC (1998). Detection of weak estrogenic flavonoids using a recombinant yeast strain and a modified MCF7 cell proliferation assay. *Chem Res Toxicol.* 11(6):622-9.

TECHNICAL NOTES

The QBlue Cell Viability assay kit has been specially optimized and formulated to provide a sensitive, convenient and robust assay for cell proliferation and cytotoxicity. Key features of the kits are as follows:

Safe. Non-radioactive assay (cf. ^3H thymidine incorporation assay).

Sensitive and accurate. As low as 100 cells can be accurately quantified.

Saves time. High-throughput assay in 96-well and 384-well plates allows simultaneous processing of a large number of samples.

Homogeneous and convenient. A single reagent and "mix-incubate-measure" type assay. No wash and reagent transfer steps are involved.

Robust and amenable to HTS. Z' factors of 0.6 to 0.9 are routinely observed in 96-well and 384-well plates. Can be readily automated on HTS liquid handling systems.

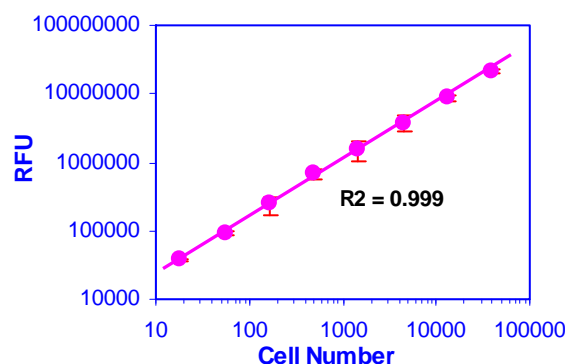


Figure 1. Linear relationship between QBlue fluorescence and cell number. HEK293 cells were serially diluted and treated with QBlue Reagent in a 384-well plate. Fluorescence intensity was linear ($R^2 = 0.999$) to the cell number. The detection limit estimated from the blank controls was 100 cells.

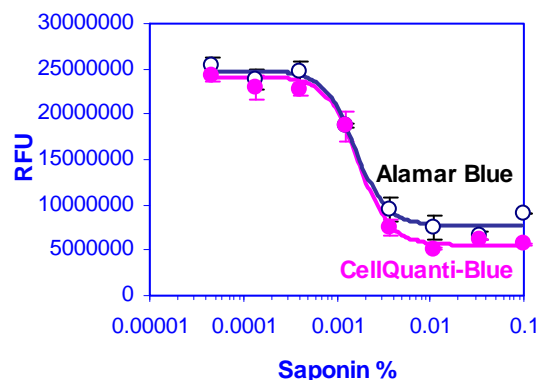


Figure 2. Dose-dependent cytotoxicity of saponin in HEK293 cells. HEK293 cells were plated at 15,000 cells per well (40 μL). Cells were treated with varying concentrations of saponin. After culture overnight, 5 μL QBlue Reagent (solid circles) was added. AlamarBlue (open circles) was also tested for comparison. Following a three-hour incubation, fluorescence intensity was measured on a Molecular Devices L.J.L. Analyst. Data represent mean \pm SD ($n = 3$). The IC_{50} values of saponin for QBlue (signal: Background = 4.8-fold) and Alamar Blue (signal: Background = 3.3-fold) were 0.0016 wt% and 0.0017 wt%, respectively.