invitrogen™

alamarBlue® Cell Viability Reagent

Catalog nos. DAL1025, DAL1100

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage*	Stability
alamarBlue® reagent	25 mL (Cat. no. DAL1025) 100 mL (Cat. no. DAL1100)	10X, ready-to-use solution	• 2–8˚C • Protect from light	When stored as directed this kit is stable until the expiration date printed on the product.
Number of assays: Sufficient reagent is supplied for 25 microplates (2,500 assays) for Cat. no. DAL1025 or 100 microplates (10,000 assays) for Cat. no. DAL1100 based on the protocol below.				
Approximate fluorescence excitation/emission maxima: 540–570/580–610 nm. If using an instrument with a monochrometer, use 560/590 nm. Peak excitation/emission are 570/585 nm.				
Absorbance maxima: 570 nm (additionally monitor 600 nm as a reference).				

Introduction

Cell health can be monitored by numerous methods. Plasma membrane integrity, DNA synthesis, DNA content, enzyme activity, presence of ATP, and cellular reducing conditions are known indicators of cell viability and cell death. alamarBlue® cell viability reagent functions as a cell health indicator by using the reducing power of living cells to quantitatively measure the proliferation of various human and animal cell lines, bacteria, plant, and fungi allowing you to establish relative cytotoxicity of agents within various chemical classes. When cells are alive they maintain a reducing environment within the cytosol of the cell. Resazurin, the active ingredient of alamarBlue® reagent, is a non-toxic, cell permeable compound that is blue in color and virtually non-fluorescent. Upon entering cells, resazurin is reduced to resorufin, a compound that is red in color and highly fluorescent (Figure 1). Viable cells continuously convert resazurin to resorufin, increasing the overall fluorescence and color of the media surrounding cells.

alamarBlue® cell viability reagent is used to assess cell viability by simply adding the 10X, ready-to-use solution to mammalian or bacterial cells in culture media (Figure 2). There is no requirement to aspirate media from cells or place cells in minimal media. Consequently, alamarBlue® reagent can easily be used in a single tube or microtiter plate format in a "no-wash" fashion. Simply add alamarBlue® reagent as 10% of the sample volume (i.e., add 10 µL alamarBlue® reagent to 100 µL sample), followed by a 1–4 hours incubation at 37ºC. Longer incubation times may be used for greater sensitivity without compromising cell health (Figure 3 and see **Frequently Asked Questions**). The resulting fluorescence is read on a plate reader or fluorescence spectrophotometer. Alternatively, the absorbance of alamarBlue® reagent can be read on a spectrophotometer. Finally, results are analyzed by plotting fluorescence intensity (or absorbance) versus compound concentration.

alamarBlue® reagent can detect as few as 50 cells per well in a 96-well plate. To evaluate the sensitivity of alamarBlue® reagent, a serial dilution of HUVEC cells was performed in a black, clear-bottom 96-well plate. Cells were then treated with alamarBlue® reagent and the fluorescence was measured 40 minutes and 18 hours later. After 40 minutes, the fluorescence intensity of alamarBlue® reagent was directly proportional to cell number in the range of 500–50,000 cells. After 18 hours, the fluorescence intensity of alamarBlue® reagent was directly proportional to cell number in the range of 50–5,000 cells, leading to more sensitive detection (Figure 3).

Figure 1A. alamarBlue® works as a cell viability and proliferation indicator through the conversion of resazurin to resorufin. Resazurin, a non-fluorescent indicator dye, is converted to highly red fluorescent resorufin via reduction reactions of metabolically active cells. The amount of fluorescence produced is proportional to the number of living cells.

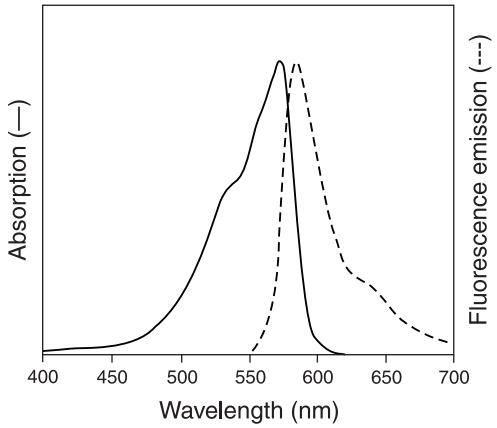

Figure 1B. Absorbance and fluorescence emission spectra of resorufin.

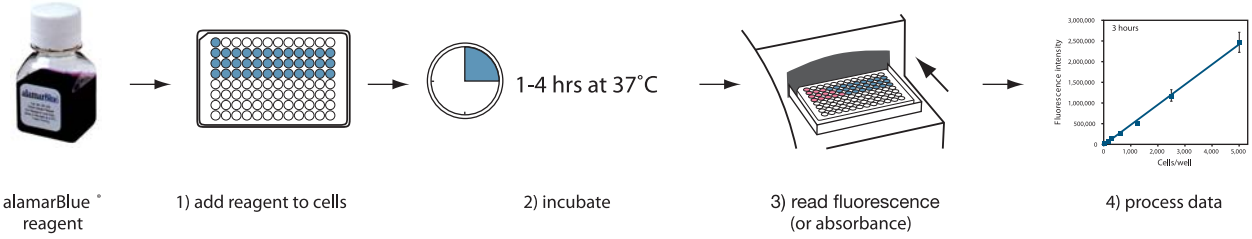

Figure 2. alamarBlue® cell viability assay protocol. A 96-well plate containing the cells and the compounds to be tested is prepared using standard methods. alamarBlue® reagent is added directly to each well, the plates are incubated at 37°C to allow cells to convert resazurin to resorufin, and the fluorescence (or absorbance) signal is measured. Results are evaluated by plotting the fluorescent (or absorbance) signal versus compound concentration. This depicts an assay carried out in a 96-well plate, but the procedure is readily adaptable to other formats as well (including 384-well plates and tubes of various volumes). If tubes are used, the sample is transferred to a cuvette prior to spectrophotometric analysis.

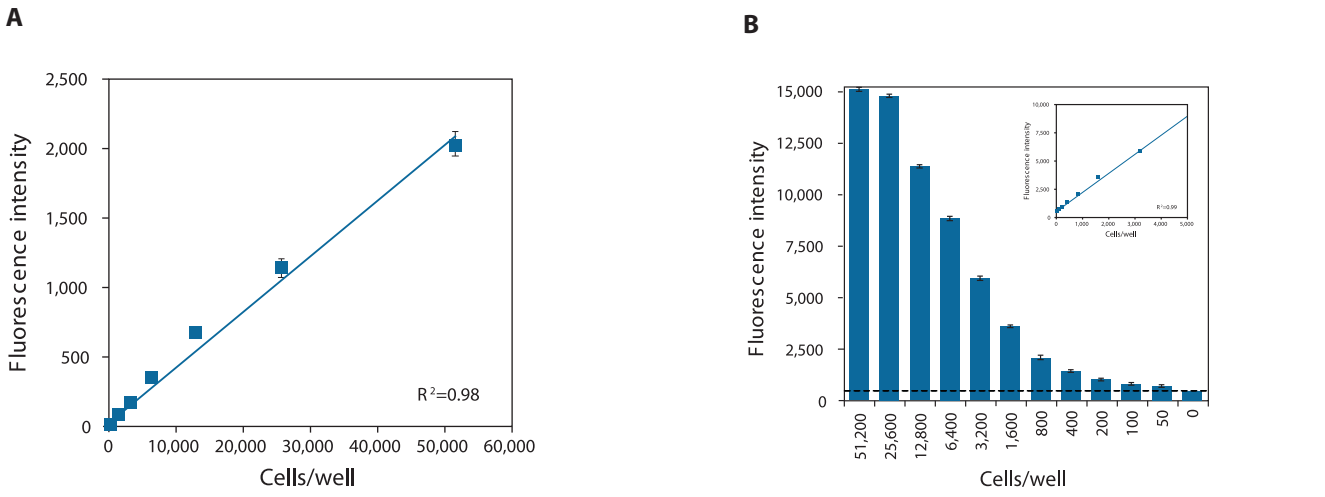

Figure 3. Linearity and sensitivity of alamarBlue® reagent on HUVEC cells. A) alamarBlue® reagent is linear over the range from ~500 to 50,000 cells after a 40-minute incubation of cells with reagent. **B)** The same 96 well plate of cells was read after 18-hour incubation and shows the sensitivity of alamarBlue® reagent. The horizontal line at ~450 RFU represents the background fluorescence in the experiment, which was calculated as three times the standard deviation of the "no cell" control. The inset graph shows alamarBlue® to be linear over the range from 50 to 5,000 cells/well after 18 hour incubation of cells with reagent. Error bars are shown as ±SEM.

Advantages of alamarBlue® Reagent

The alamarBlue® assay offers the following advantages over conventional spectrophotometric or radioactive cytotoxicity assays:

Time Savings: Ready-to-use, add-and-read format reduces sample handling steps.

Multiple Formats: Suitable for use with eukaryotic or prokaryotic cell types, single tube or microtiter plate assay, and data recorded using fluorescence or absorbance measurements.

Endpoint or Kinetic Reads: Perform endpoint or kinetic assays for analysis of cellular viability or compound toxicity.

*Convenient: S*upplied as a ready-to-use solution for high-throughput screening in 96- or 384-well formats.

Multiplexing: Compatible for multiplexing with other assay methods such as CyQUANT® NF Cell Proliferation Assay Kit (Cat. no. C35006) for detecting nucleic acid as a viability indicator (Figure 4).

Note: You may need to empirically determine the ability to multiplex with CyQUANT® NF Cell Proliferation Assay Kit based on the cell type used.

Inexpensive: Invitrogen's alamarBlue® reagent is formulated as 10X as opposed to 5X which allows you to perform twice the assays as compared to competitor's products.

Safe: Non-toxic reagent allows real-time monitoring of cell metabolism and viability[1] and does not use any hazardous solvents, or require disposal of scintillation cocktail and radioactive waste.

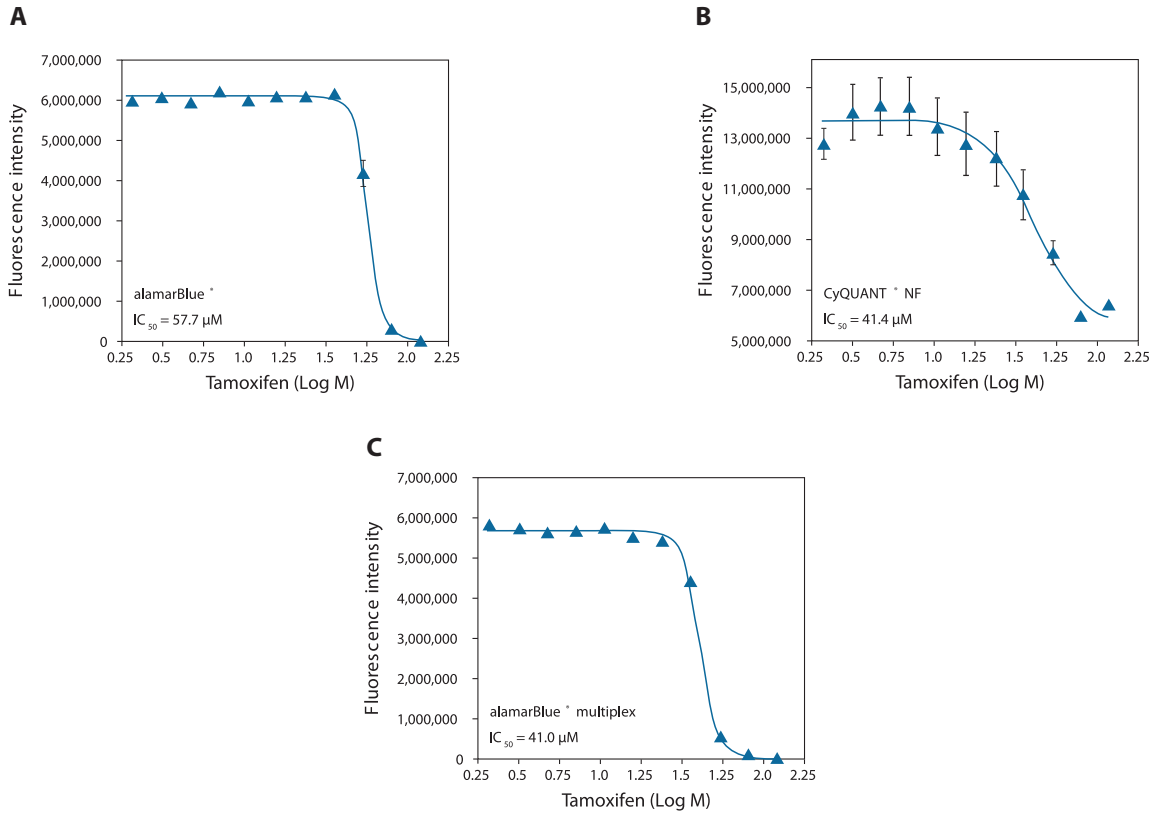

Figure 4. Multiplexing of alamarBlue® reagent with CyQUANT® NF. HepG2 cells were incubated with tamoxifen for 24 hours prior to the addition of **A)** alamarBlue® reagent **B)** CyQUANT® NF, or **C)** alamarBlue® reagent and CyQUANT® NF. The indicated IC_{50} values demonstrate that alamarBlue® reagent can be multiplexed with other cytotoxic indicators and still give accurate results.

Before You Begin

Materials Required but Not Provided

- Mammalian or bacterial cells in appropriate medium
- Appropriate 96- or 384-well plates
- Optional: 3% SDS in phosphate buffered saline (PBS), pH 7.4

Preparing Cells

Mammalian Cells—Adherent: Plate mammalian cells in a cell culture flask or dish, and allow cells to adhere and grow for approximately 4–24 hours at 37°C and 5% CO_2 before proceeding with the assay.

Mammalian Cells—Suspension: Plate mammalian cells in a cell culture flask or dish, and use cells immediately for the assay or allow cells to grow for up to 24 hours at 37°C and 5% CO_2 before proceeding with the assay.

Bacterial Cells: For details, see references 2 and 3.

Notes

- alamarBlue® reagent is stable to multiple freeze/thaw cycles and its activity is not affected if the reagent is frozen.
- Review the **Frequently Asked Questions** (page 6) before starting the protocol.

General Guidelines

- Cell types assayed with alamarBlue® reagent include mammalian, bacterial[2] (including biofilms[3]), plant,[4] and fish cells.[5] More specifically alamarBlue® reagent has been tested on hepatocytes, such as HepG2 cells, as well as cells of primary origin.[5]
- Be sure to include appropriate assay controls. To minimize experimental errors, we recommend making measurements from a minimum of 4–8 replicates of experimental and no-cell control samples.
- You may need to determine the plating density and incubation time for the alamarBlue® assay for each cell type and use conditions such that the assay is in the linear range.
- If you plan to use longer incubation time (overnight), be sure to maintain sterile conditions during reagent addition and incubation to avoid microbial contaminants. Contaminated cultures will yield erroneous results as microbial contaminants also reduce alamarBlue® reagent.
- Fetal bovine serum (FBS) and bovine serum albumin (BSA) cause some quenching of fluorescence. We recommend using the same serum concentration in controls to account for this quenching. Other media components, such as phenol red do not interfere with the assay.

Experimental Protocols

alamarBlue® Cell Viability Protocol

Optional: Treat cells with the test compound 24–72 hours prior to performing the alamarBlue® cytotoxicity assay.

1.1 Add 1/10th volume of alamarBlue® reagent directly to cells in culture medium as described in Table 2.

Table 2. Assay volumes.

Format	Volume of cells + medium	Volume of 10X alamarBlue® to add
Cuvette	1 mL	100 µL
96-well plate	100 µL	10 µL
384-well plate	40 µL	4 µL

1.2 Incubate for 1 to 4 hours at 37°C in a cell culture incubator, **protected from direct light**.

Note: Sensitivity of detection increases with longer incubation times. For samples with fewer cells, use longer incubation times of up to 24 hours.

1.3 Record results using fluorescence or absorbance as follows:

Fluorescence: Read fluorescence using a fluorescence excitation wavelength of 540–570 nm (peak excitation is 570 nm). Read fluorescence emission at 580–610 nm (peak emission is 585 nm).

Absorbance: Monitor the absorbance of alamarBlue® at 570 nm, using 600 nm as a reference wavelength (normalized to the 600 nm value).

Note: Fluorescence mode measurements are more sensitive. When fluorescence instrumentation is unavailable, monitor the absorbance of alamarBlue® reagent. Assay plates or tubes can be wrapped in foil, stored at 4°C, and read within 1–3 days without affecting the fluorescence or absorbance values.

1.4 **Optional:** Add 50 µL 3% SDS directly to 100 µL of cells in alamarBlue® reagent to stop the reaction.

Data Processing

Fluorescence

2.1 Plot fluorescence intensity versus concentration of the test compound.

2.2 **Optional:** Subtract the average fluorescence values of the cell culture medium alone (background) from the fluorescence values of experimental wells.

Absorbance

2.3 Subtract the average 600 nm absorbance values of the cell culture medium alone (background) from the 570 nm absorbance values of experimental wells.

2.4 Plot background subtracted 570 nm absorbance versus concentration of the test compound.

Comparison to Other Assay Methods

alamarBlue® reagent was tested against other cell viability and proliferation assays and shown to provide similar pharmacological data (Figures 4 and 5).

Frequently Asked Questions:

General Questions

Q: How does alamarBlue® work?
A: Healthy living cells maintain a reducing state within their cytosol. This "reducing potential" of cells converts alamarBlue® reagent into a detectable fluorescent (or absorbent) product.

Q: Is alamarBlue® reagent toxic?
A: No. alamarBlue® reagent is a safe, non-toxic reagent to both the sample and user.

Q: Does alamarBlue® reagent need reconstitution?
A: No, alamarBlue® reagent is supplied as a 10X, ready-to-use solution.

Q: Can I use alamarBlue® reagent with suspension cells too?
A: Yes. alamarBlue® reagent works on adherent and suspension mammalian cells.

Q: Can I use alamarBlue® reagent with non-mammalian cells, such as bacteria?
A: Yes, alamarBlue® reagent has been shown to work with bacterial[2] and plant cells.[4]

Q: alamarBlue® reagent is not the most expensive cytotoxicity indicator on the market, does that mean it doesn't work as well as other reagents?
A: Actually, alamarBlue® reagent is comparable to other often more expensive cytotoxicity indicators. See Figure 5 for comparison data.

Q: Since alamarBlue® is an absorbance or fluorescence readout, is it as sensitive as a luminescence product?

A: alamarBlue® reagent is sensitive enough to detect less than 50 mammalian cells in a single well of a 96-well plate. See Table 3 and Figure 3 for details.

Storage Questions

Q: What if I left the alamarBlue® stock reagent at room temperature, overnight?
A: The reagent is stable for up to 12 months when stored at room temperature (~22°C).

Q: I accidentally froze the alamarBlue® stock reagent, can I still use it?
A: Yes. alamarBlue® reagent is stable to multiple freeze/thaw cycles. Be sure to heat the reagent in a 37°C water bath and mix the reagent to ensure a homogenous solution before use.

Q: Do I need to protect alamarBlue® reagent from light?
A: Yes, alamarBlue® reagent is very slowly converted into a fluorescent product over time, when exposed to light, thus leading to high background values. Store the reagent, protected from light.

Methods Questions

Q: What is the optimal incubation time and temperature of cells with alamarBlue® reagent?
A: Incubate the cells with alamarBlue® reagent for 1–4 hours at 37°C. For more sensitive detection with low cell numbers, increase the incubation time for up to 24 hours. See Figure 6 for details.

Q: Can you incubate cells with alamarBlue® reagent overnight?
A: Yes. However, signals from higher cell density samples may have "saturated," which means the linearity of reagent may have reached a plateau. If this occurs, decrease the incubation time.

Q: What if I don't have an instrument suitable for reading fluorescence?
A: The absorbance of alamarBlue® reagent also changes depending on cell viability and proliferation. Therefore, simply monitor the absorbance of the reagent at 570 nm, while using 600 nm as a reference wavelength.

Q: Is alamarBlue® assay strictly an endpoint assay?
A: No. While alamarBlue® can be used as a terminal readout of a population of cells, the reagent can also be used to continuously monitor cell viability and proliferation in real time. Since alamarBlue® reagent is non-toxic, you can incubate cells with reagent and monitor fluorescence (or absorbance) over time on the same sample.

Troubleshooting Questions

Q: What is the problem for observing high background fluorescence values?
A: The reagent may be breaking down due to exposure to light. Be sure to store alamarBlue® reagent in the dark and do not expose the reagent to direct light for long periods of time.

Q: Why are the fluorescence values so low in intensity?
A: Try increasing the incubation time of cells with alamarBlue® reagent, changing the instrument's "gain" setting, and checking the instrument filter/wavelength settings. Make sure to have positive controls (living cells) in the experimental design for troubleshooting.

Q: Why are the fluorescence values so high that they are beyond the linear range of the instrument?
A: Try decreasing the incubation time or reducing the number of cells used in the experiment.

References

1. Invest Ophthalmol Vis Sci 38, 1929 (1997); **2.** Infect Immun 65, 3193 (1997); **3.** J Antimicrob Chemother 57, 1100 (2006); **4.** Phytochem Anal 12, 340 (2001); **5.** Anal Biochem 344, 76 (2005).

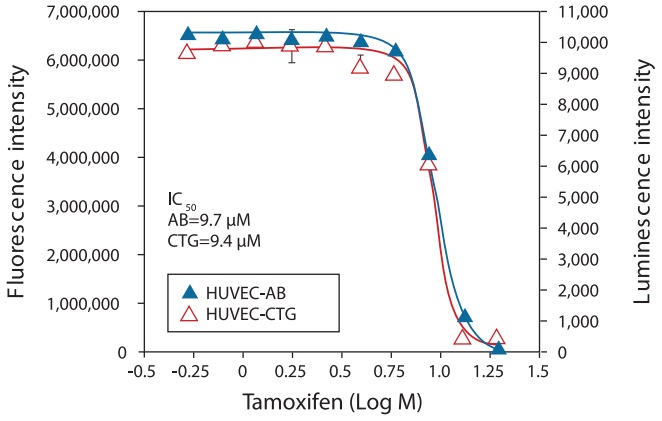

Figure 5. Comparison of alamarBlue® reagent with CellTiter® Glo. HUVEC cells were treated with tamoxifen for 24 hours prior to performing the cytotoxicity assays. The alamarBlue® (AB) and CellTiter® Glo (CTG) assays were performed according to the manufacturer's instructions. alamarBlue® assay gave nearly identical results at a fraction of the cost of CellTiter® Glo.

Table 3. Limit of alamarBlue® reagent detection in a 96-well plate.

Cell Line	Detection Limit*		
	40 minutes	3 hours	Overnight (18 hours)
HASmC (primary)	~195	<48	<48
HUVEC (primary)	~781	<48	<48
HPAEC (primary)	~390	<48	<48
HEK 293	~390	~390	<48
HepG2	~1563	~195	<48
SH-SY5Y	~1563	<48	<48

* values are given as the number of cells/well.

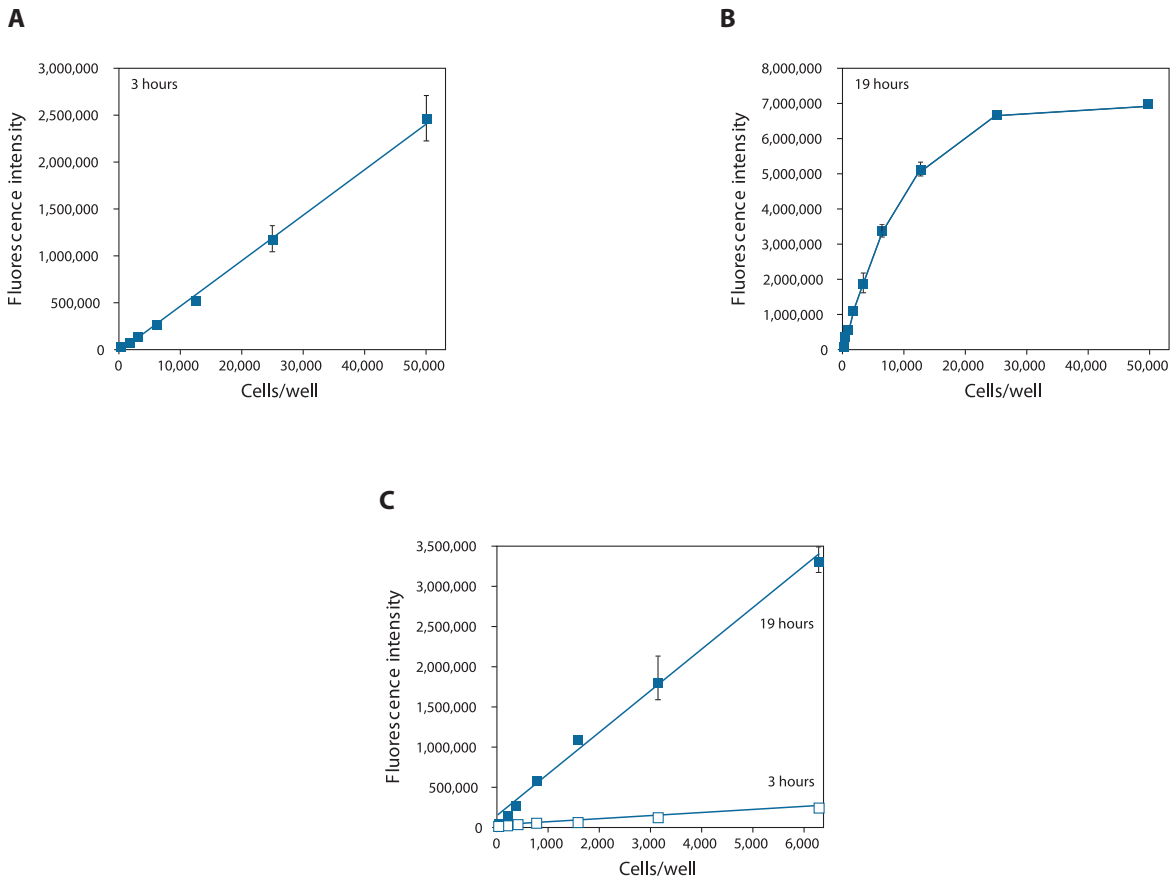

Figure 6. Effect of incubation time on fluorescence signal formation with alamarBlue® reagent. SH-SY5Y cells were plated in a 96-well plate at different cell densities. alamarBlue® reagent was added to the plate and allowed to incubate at 37ºC for **A)** 3 hours, **B)** 19 hours, **C)** data from 3 hours and 19 hours is re-plotted to show the difference in alamarBlue® sensitivity when working with small numbers of cells per well, N = 4, ±SEM.

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
DAL1025	alamarBlue® cell viability reagent, 10X	25 mL
DAL1100	alamarBlue® cell viability reagent, 10X	100 mL

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Order Fax: (800) 438-0228

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