

# alamarBlue™ Cell Viability Assay Reagent

88951 88952

2502.0

Number	Description
88951	<b>alamarBlue Cell Viability Assay Reagent</b> , 25mL, ready-to-use solution sufficient for 2500 reactions in a 96-well plate or 10,000 reactions in a 384-well plate
88952	<b>alamarBlue Cell Viability Assay Reagent</b> , 100mL, ready-to-use solution sufficient for 10,000 reactions in a 96-well plate or 40,000 reactions in a 384-well plate

**Storage:** Upon receipt store at 4°C protected from light. Product shipped at ambient temperature.

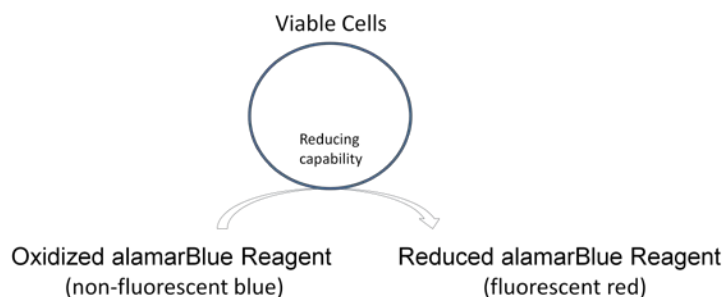
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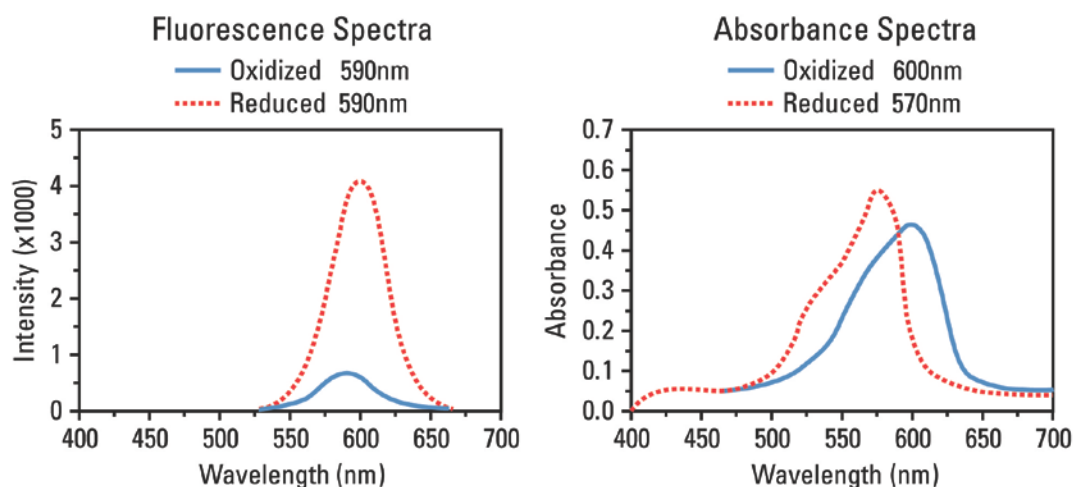
## Introduction

The Thermo Scientific alamarBlue Cell Viability Assay Reagent is used to quantitatively measure the viability of mammalian cell lines, bacteria and fungi by incorporating a rapid, sensitive and reliable fluorometric/colorimetric growth indicator. The alamarBlue Reagent allows for viability studies of chemical inhibitors on tumor cell lines and toxicology research to establish baseline data for predicting the toxicity of related novel agents by comparing such baseline data with known *in vivo* toxicity.<sup>1,2</sup> Additionally, tissue engineering scientists can monitor viability in real time when performing a variety of tissue engineering applications.<sup>3,4</sup>

Viable cells maintain a reducing environment within their cytoplasm. The alamarBlue Reagent is an oxidized form of redox indicator that is blue in color and non-fluorescent. When incubated with viable cells, the reagent changes color from blue to red and becomes fluorescent (Figure 1). This change can be detected using fluorescence or absorbance measurement instruments (see Procedure Summary Section) (Figure 2 and 3).

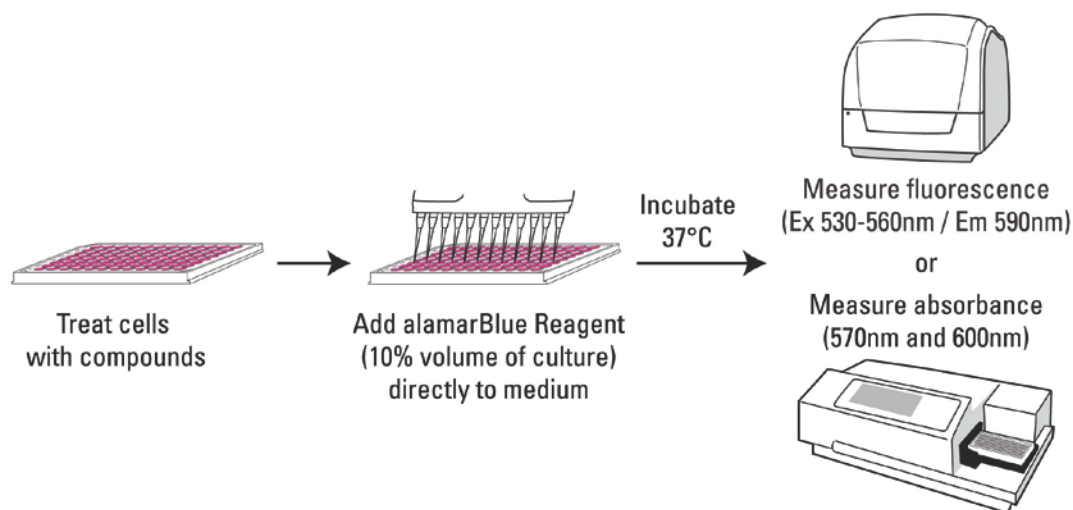


**Figure 1. Representative schematic of Thermo Scientific alamarBlue Cell Viability Reagent undergoing reduction within the cells.**



**Figure 2. Fluorescence (left) and absorbance (right) spectra of Thermo Scientific alamarBlue Reagent in oxidized and reduced states.**

## Procedure Summary



**Figure 3. Procedure summary schematic.**

## Important Product Information

- Store the assay protected from light.
- Do not use the assay with media containing reagents with high redox potential (e.g., DTT).
- Procedures are written for 96-well plates; for 384-well plates, reduce all volumes by four-fold.

## Additional Materials Required

- Sterile, ultrapure water
- Cultured cell line
- Tissue culture-compatible 96- or 384-well plates compatible with fluorometer and absorbance reading (clear or solid bottom)

- Multichannel pipette
- Fluorescent reader with excitation of 530-560nm and emission of 590nm
- Spectrophotometer plate reader capable of reading 570/600nm absorbance

**Note:** Alternative wavelengths of 570/630nm or 540/600nm can be used; do not use a 540/630nm reading.

- Positive control: 100% reduced form of alamarBlue Reagent. To prepare the reduced reagent form, autoclave alamarBlue Reagent in culture media (1 volume of alamarBlue Reagent: 10 volumes of mammalian cell culture media containing serum) for 15 minutes of liquid autoclave cycle.

**Note:** Do not autoclave concentrated alamarBlue Reagent or alamarBlue Reagent in phosphate-buffered saline (PBS); 100% reduced alamarBlue Reagent is not stable in PBS.

## Determination of Optimum Incubation Time and Cell Number

**Note:** Cellular response within the assay is highly dependent on the incubation time and the number of cells plated. For best results, determine the optimal incubation time and plating density for each different cell line.

1. Prepare a log phase growth of cells in 100µL of media with cell numbers above and below the cell density expected to be used.

**Note:** Include a negative control of only medium without cells to determine background signal. Include a positive control of 100µL of 100% reduced alamarBlue Reagent without cells.

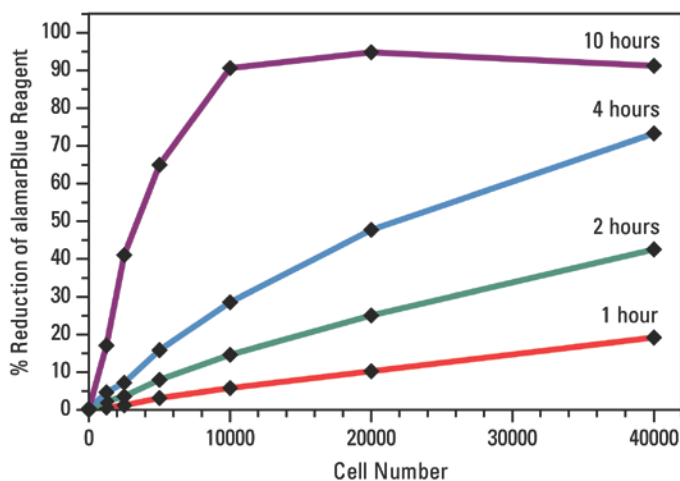
2. Add 10µL of alamarBlue Reagent in an amount equal to 10% of the volume in the well. For the positive control well, add 10µL of sterile, ultrapure water.
3. Incubate the plate in an incubator at 37°C, 5% CO<sub>2</sub>.
4. Remove the plate and measure fluorescence with excitation wavelength at 530-560nm and emission wavelength at 590nm (refer to Step 5 for calculation) or absorbance at a wavelength of 570nm and 600nm (refer to Step 6 for calculation).

**Note:** Two alternative absorbance wavelength pairs of 570/630nm or 540/600nm can be used (refer to Step 6 for calculation).

5. To calculate the % Reduction of alamarBlue Reagent using fluorescence readings:

$$\% \text{ Reduction of alamarBlue Reagent} = \frac{(\text{Experimental RFU value} - \text{Negative control RFU value})}{(\text{100\% reduced positive control RFU value} - \text{Negative control RFU value})} \times 100$$

**Figure 4. Graph showing the percentage reduction of Thermo Scientific alamarBlue Reagent at different cell numbers and incubation hours.** Serial dilutions of CHO-K1 cells were plated at 100µL/well in a 96-well plate and incubated at 37°C, 5% CO<sub>2</sub>. The alamarBlue Reagent (10µL/well) was added and the cells were measured at 545nm<sub>Ex</sub>/590nm<sub>Em</sub> using a Thermo Scientific Varioskan Flash Multimode Plate Reader.



6. From Figure 4, if the desired incubation time with alamarBlue Reagent was 4 hours, any plating density from 5000 to 40,000 cells/mL could be used and expected to produce a reaction within the linear range for that incubation period. However, if the intent is to incubate for 10 hours, the reaction could be expected to be within the linear range if plated at 1250 to 10,000 cells/mL for this cell line. There is no longer a linear relationship with the percentage of reduction above 10,000 cells/mL.

**Note:** Microbial contaminants will also reduce alamarBlue Reagent and yield erroneous results if contaminated cultures are tested by this method.

To calculate the % Reduction of alamarBlue Reagent using absorbance readings:

$$\% \text{ Reduction of alamarBlue Reagent} = \frac{(E_{\text{oxi}600} \times A_{570}) - (E_{\text{oxi}570} \times A_{600})}{(E_{\text{red}570} \times C_{600}) - (E_{\text{red}600} \times C_{570})} \times 100$$

$E_{\text{oxi}570}$  = molar extinction coefficient (E) of oxidized alamarBlue Reagent at 570nm = 80586

$E_{\text{oxi}600}$  = E of oxidized alamarBlue Reagent at 600nm = 117216

$A_{570}$  = absorbance of test wells at 570nm

$A_{600}$  = absorbance of test wells at 600nm

$E_{\text{red}570}$  = E of reduced alamarBlue at 570nm = 155677

$E_{\text{red}600}$  = E of reduced alamarBlue at 600nm = 14652

$C_{570}$  = absorbance of negative control well (media, alamarBlue Reagent, no cells) at 570nm

$C_{600}$  = absorbance of negative control well (media, alamarBlue Reagent, no cells) at 600nm

**Note:** Use different values for E for alternative wavelengths of 540/600nm and 570/630nm.

$E_{\text{oxi}540}$  = E of oxidized alamarBlue Reagent at 540nm = 47619

$E_{\text{oxi}630}$  = E of oxidized alamarBlue Reagent at 630nm = 34798

$E_{\text{red}540}$  = E of reduced alamarBlue Reagent at 540nm = 104395

$E_{\text{red}630}$  = E of reduced alamarBlue Reagent at 630nm = 5494

## Cell Viability Assay with Chemical Compounds

1. Harvest log phase-growth cells. Plate cells according to previously determined optimum cell density.

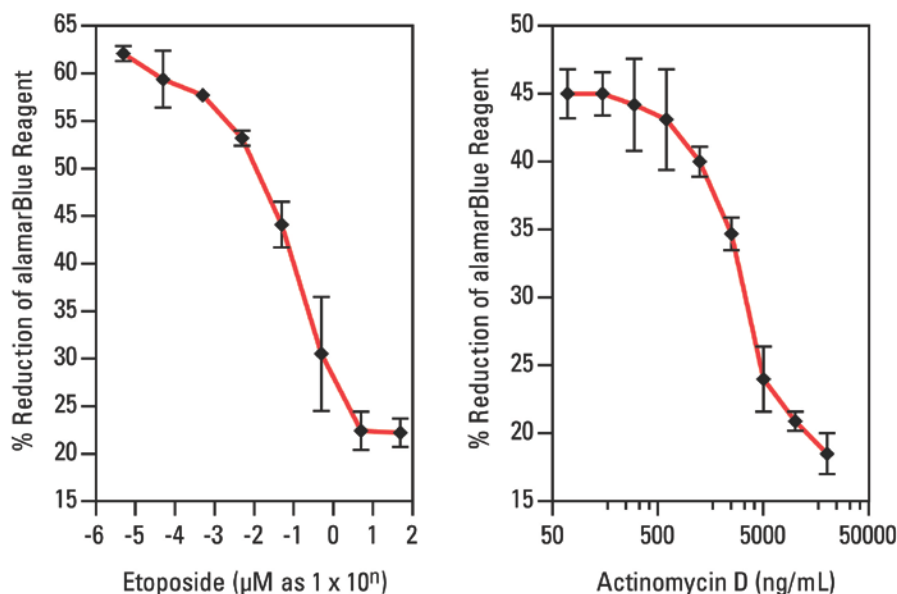
**Note:** Include (1) a medium control without cells to determine absorbance of negative control; (2) an untreated cell control to serve as a vehicle control by adding the same solvent used to dissolve the chemical compound or growth factor; (3) a 100% reduced alamarBlue positive control.

2. Add a chemical compound and vehicle control to the well.
3. Incubate cells for the required time in an incubator at 37°C, 5% CO<sub>2</sub>.
4. Add 10µL of alamarBlue Reagent in an amount equal to 10% of the volume in the well. For the positive control well, add 10µL of sterile, ultrapure water.
5. Incubate cells for the pre-determined time in an incubator at 37°C, 5% CO<sub>2</sub>.
6. Remove the plate and measure fluorescence with excitation wavelength at 530-560nm and emission wavelength at 590nm (refer to Step 7 or 8 for calculation). Alternatively, measure absorbance at a wavelength of 570nm and 600nm (refer to Step 8 for calculation).

**Note:** Alternative wavelengths of 570/630nm or 540/600nm can be used (refer to Step 7 for calculation).

7. To calculate the % Reduction of alamarBlue Reagent using fluorescence-based readings:

$$\% \text{ Reduction of alamarBlue Reagent} = \frac{(\text{Experimental RFU value} - \text{Negative control RFU value})}{(100\% \text{ reduced positive control RFU value} - \text{Negative control RFU value})} \times 100$$



**Figure 5. Determination of cell viability using Thermo Scientific alamarBlue Cell Viability Reagent.** HEK-293 or CHO-K1 cells were plated in a 96-well plate and exposed to various concentrations of Etoposide and Actinomycin D, respectively. Cells were loaded with alamarBlue Reagent, incubated at 37°C, 5% CO<sub>2</sub>, and the plates were measured at 545nm<sub>Ex</sub>/590nm<sub>Em</sub> using a Varioskan™ Flash Multimode Plate Reader.

9. To calculate the % Difference between treated and untreated control cells based on fluorescence readings:

$$\% \text{ Difference between treated and untreated cells} = \frac{\text{Experimental RFU value with chemical compound}}{\text{Untreated cell control RFU value}} \times 100$$

10. To calculate the percentage reduction of alamarBlue Reagent using absorbance readings:

$$\% \text{ Reduction of alamarBlue Reagent} = \frac{(E_{\text{oxi}600} \times A_{570}) - (E_{\text{oxi}570} \times A_{600})}{(E_{\text{red}570} \times C_{600}) - (E_{\text{red}600} \times C_{570})} \times 100$$

E<sub>oxi</sub>570 = E of oxidized alamarBlue Reagent at 570nm = 80586

E<sub>oxi</sub>600 = E of oxidized alamarBlue Reagent at 600nm = 117216

A<sub>570</sub> = absorbance of test wells at 570nm

A<sub>600</sub> = absorbance of test wells at 600nm

E<sub>red</sub>570 = E of reduced alamarBlue Reagent at 570nm = 155677

E<sub>red</sub>600 = E of reduced alamarBlue Reagent at 600nm = 14652

C<sub>570</sub> = absorbance of negative control well (media, alamarBlue Reagent, no cells) at 570nm

C<sub>600</sub> = absorbance of negative control well (media, alamarBlue Reagent, no cells) at 600nm

**Note:** Use different values for E for alternative wavelengths of 540/600nm and 570/630nm.

E<sub>oxi</sub>540 = E of oxidized alamarBlue Reagent at 540nm = 47619

E<sub>oxi</sub>630 = E of oxidized alamarBlue Reagent at 630nm = 34798

E<sub>red</sub>540 = E of reduced alamarBlue Reagent at 540nm = 104395

E<sub>red</sub>630 = E of reduced alamarBlue Reagent at 630nm = 5494

## Troubleshooting

Problem	Possible Cause	Solution
Low fluorescence value	Low instrument gain setting	Adjust the instrument's gain setting
	Improper instrument filter/ wavelength	Verify the instrument's filter/wavelength setting
	Short incubation time	Increase incubation time
	Low number of cells	Increase cell number
High fluorescence value	High instrument gain setting	Adjust the instrument's gain setting
	Improper instrument filter/ wavelength	Verify the instrument's filter/wavelength setting
	Long incubation time	Decrease incubation time
	High number of cells	Decrease cell number
	Bacterial contamination	Identify and remove the contamination source
	AlamarBlue Reagent was exposed to direct light for extended time	Purchase new AlamarBlue Reagent after comparing current with previous readings of negative control

## Related Thermo Scientific Products

<b>88953-4</b>	<b>Pierce™ LDH Cytotoxicity Assay</b>
<b>8410000*</b>	<b>Live Cell Green</b>
<b>8410200*</b>	<b>Live Cell Violet</b>
<b>8410100*</b>	<b>Live Cell Blue</b>
<b>K0200011*</b>	<b>Cell Viability Kit</b>
<b>51119000*</b>	<b>Multiskan™ FC Microplate Photometer</b>
<b>51119100*</b>	<b>Multiskan FC Microplate Photometer with incubator</b>
<b>5250030*</b>	<b>Varioskan Flash Multimode Reader with top reading</b>
<b>5250040*</b>	<b>Varioskan Flash Multimode Reader with top and bottom reading</b>
<b>5250500*</b>	<b>Varioskan Flash Multimode Reader with LumiSens Option, factory-fitted (also enabling luminometric spectral scanning)</b>
<b>5250510*</b>	<b>Varioskan Dispenser Option</b>

\* Products can be found at [www.thermoscientific.com](http://www.thermoscientific.com)

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## General Reference

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