PrestoBlue® Cell Viability Reagent

Catalog nos. A13261, A13262

Material	Sizes	Concentration	Storage	Stability
PrestoBlue® Cell Viability Reagent	25 mL (A13261) 100 ml (A13262)	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: A13261 (25 ml) 2,500 assays* (26 x 96-well plates) or 6,250 assays* (16 x 384-well plates) A13262 (100ml) 10,000 assays* (104 x 96-well plates) or 25,000 assays* (65 x 384-well plates) *calculations assume 100 µl and 40 µl final well volume for 96-well and 384-well plates respectively. If other final well volumes are used the number of assays the reagent is sufficient for will vary.				
Approximate fluorescence excitation/emission maxima: 535–560/590–615 nm.				
Absorbance maxima: 570 nm (monitor 600 nm as a reference wavelength).				

I PrestoBlue® reagent Product Overview

How does PrestoBlue® cell viability reagent work?

When cells are viable, they maintain a reducing environment within their cytosol. PrestoBlue® cell viability reagent uses that reducing ability to quantitatively measure cell proliferation, and therefore can be used to establish the relative viability of various reagents across many different cell types. PrestoBlue® reagent is a resazurin-based solution that functions as a cell viability indicator by using the reducing power of living cells to quantitatively measure the proliferation of cells. The PrestoBlue® reagent contains a cell-permeant compound that is blue in color and virtually nonfluorescent. When added to cells, the PrestoBlue® reagent is modified by the reducing environment of the viable cell and turns red in color and becomes highly fluorescent. This change can be detected using fluorescence or absorbance measurements (Figure 1).

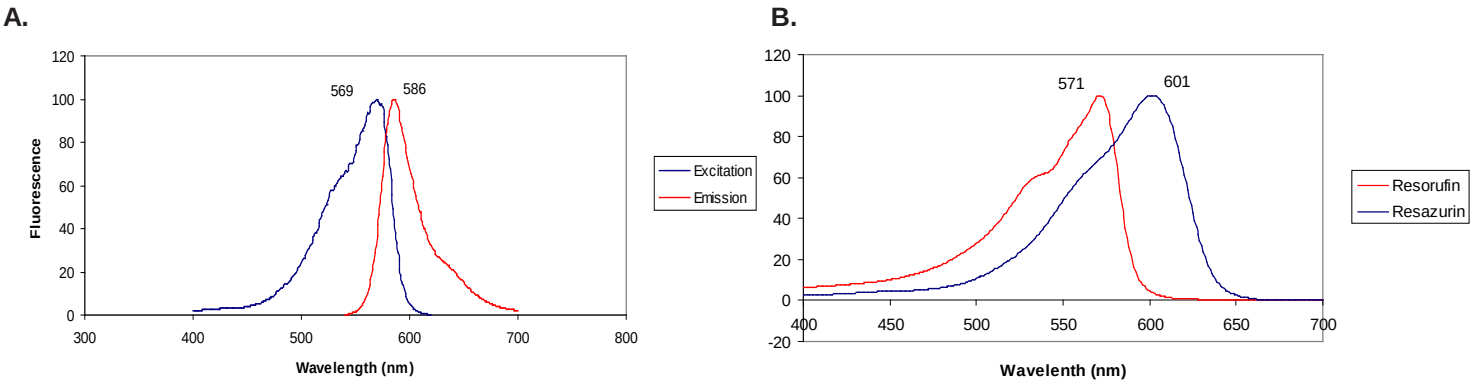


Figure 1. Reduction of PrestoBlue® reagent.
Upon entering a living cell, PrestoBlue® reagent is reduced from resazurin, a blue compound with no intrinsic fluorescent value, to resorufin which is red in color and highly fluorescent. Conversion is proportional to the number of metabolically active cells and therefore can be measured quantitatively. To measure fluorescence values use the excitation and emission peaks for resorufin (A). To measure absorbance, use the absorbance spectra for resazurin and resorufin (B).

How does PrestoBlue®reagent compare to other cell viability reagents?

PrestoBlue® cell viability reagent significantly outperforms all other resazurin-based assays on the market at the 10 minute incubation time (Figure 2) and correlates to values obtained with MTT (Figure 3) and CellTiter-Glo® (Figure 4).

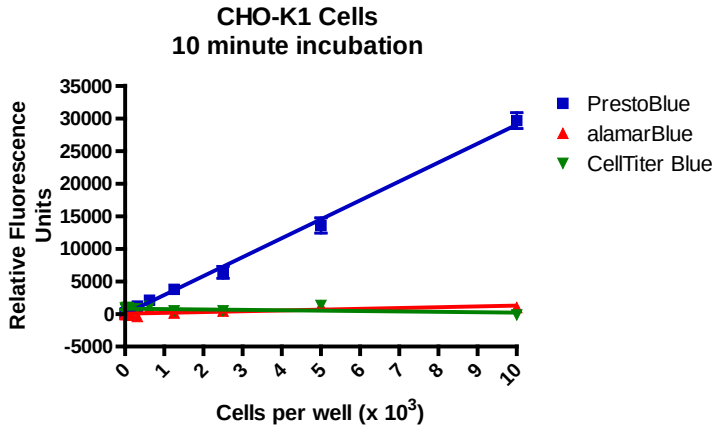
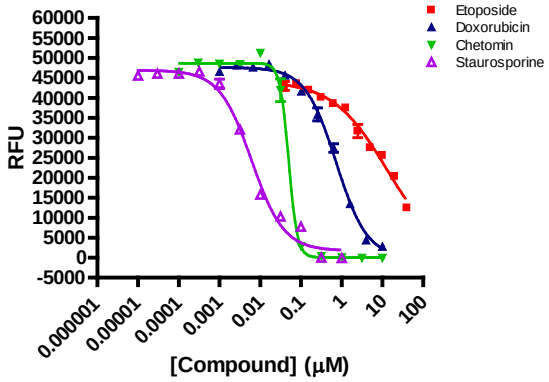
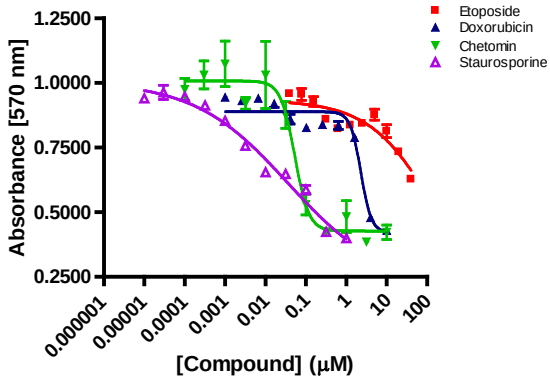


Figure 2. Comparison of PrestoBlue® reagent to other resazurin-based assays.
Serial two-fold dilutions of CHO-K1 cells were prepared in a 384-well plate and cultured overnight at 37°C/5% CO_2. PrestoBlue® reagent, alamarBlue® and CellTiter-Blue® reagents were each added to a separate section of the plate in quadruplicate, and cells were incubated at 37°C/5%CO_2 for 10 minutes before recording fluorescence.

A.



B.



C.

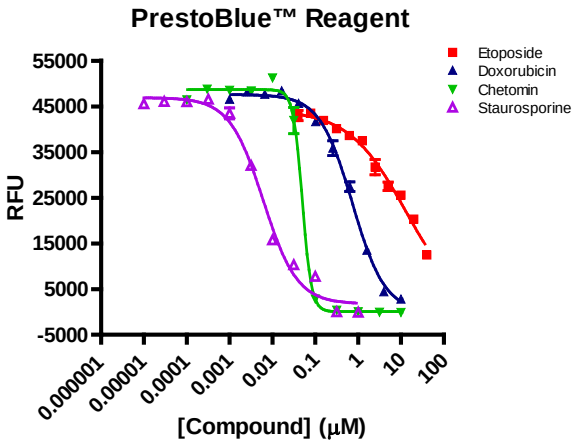
	PrestoBlue® reagent EC_{50} (µM)	MTT EC_{50} (µM)
Etoposide	12.82	220.9
Doxorubicin	0.7352	2.29
Chetomin	0.04986	0.05302
Staurosporine	0.005153	0.03626

Figure 3: Comparison of PrestoBlue® reagent to MTT. Comparable rank order potency results obtained with PrestoBlue® reagent (A) or MTT (B). U-2OS cells were plated in a 384 well plate at 2,000 cells/well. Cells were then exposed to various concentrations of Etoposide, Doxorubicin, Chetomin, or Staurosporine for 72 hours. Subsequently, cells were loaded for 10 minutes with PrestoBlue® reagent prior to assay readout or 4 hours with MTT, followed by a 16 hour solubilization step, prior to assay readout. Comparable EC_{50} values were obtained for each compound (C).

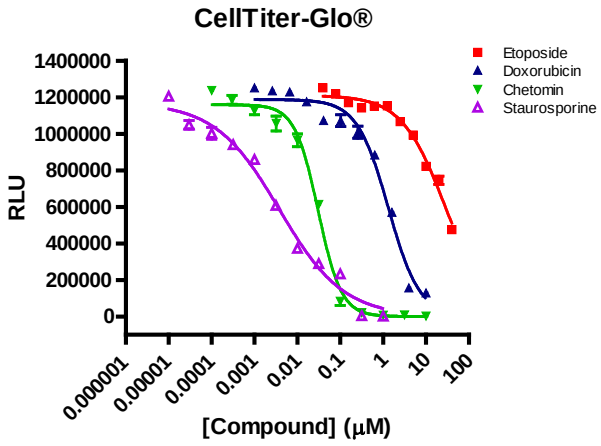
What advantages does the PrestoBlue® cell viability reagent have over a luminescence based assay?

As shown in Figure 3, comparable EC_{50} values are obtained with PrestoBlue® and CellTiter-Glo® reagents. However, PrestoBlue® reagent is a live cell assay in which cells can be further cultured after treatment, whereas the CellTiter-Glo® assay requires cell lysis (Figure 4).

A.



B.



C.

	PrestoBlue® reagent EC_{50} (µM)	CellTiter-Glo® EC_{50} (µM)
Etoposide	12.82	27.69
Doxorubicin	0.7352	1.328
Chetomin	0.04986	0.0305
Staurosporine	0.005153	0.003813

Figure 4: Comparison of PrestoBlue® reagent to CellTiter-Glo® reagent. Comparable results obtained with PrestoBlue® reagent (A) or CellTiter-Glo® reagent (B) in 10 minutes. U-2OS cells were plated in a 384 well plate at 2,000 cells/well. Cells were then exposed to various concentrations of Etoposide, Doxorubicin, Chetomin, or Staurosporine for 72 hours. Subsequently, cells were loaded for 10 minutes with PrestoBlue® reagent or CellTiter-Glo® reagent prior to assay readout. Comparable EC_{50} values were obtained for each compound (C).

How sensitive is PrestoBlue® cell viability reagent?

PrestoBlue® cell viability reagent is sensitive enough to detect 10 mammalian cells in a single well of a 384-well plate (Figure 5).

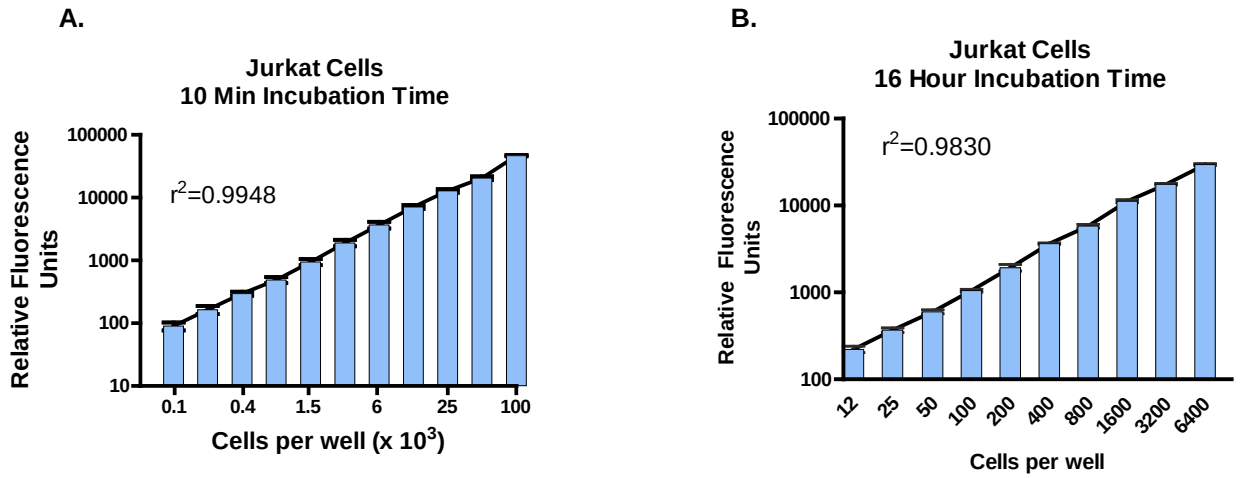


Figure 5. Effect of PrestoBlue® reagent assay incubation time on signal formation. Serial two-fold dilutions of Jurkat cells from 0–100,000 were incubated with PrestoBlue® reagent at 37°C/5% CO_2 for 10 minutes and 16 hours. For the 10-minute incubation, the signal from 98 cells was greater than the no cell control +/- 3 standard deviations (A). The 16-hour incubation, the signal from 12 cells was greater than the no cell control +/- 3 standard deviations (B).

Is the PrestoBlue® reagent assay a live-cell or end-point assay?

PrestoBlue® reagent can be either a live-cell or end-point assay. PrestoBlue® reagent allows you to develop live-cell assays for real-time monitoring of cell metabolism and viability (Figure 6) without using any hazardous solvents, or requiring disposal of scintillation cocktail and radioactive waste (1). Assayed cells can be recovered for further culturing or use in a subsequent assay. Alternatively, 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. If an end-point assay is preferred, it is possible to stop and stabilize the reaction by the adding 3% SDS (50 µL SDS for every 100 µL original culture volume is sufficient). The plate can then be stored at room temperature for up to 48 hours before recording data, provided that the contents are protected from light and covered to prevent evaporation. Cells cannot be further cultured after this step.

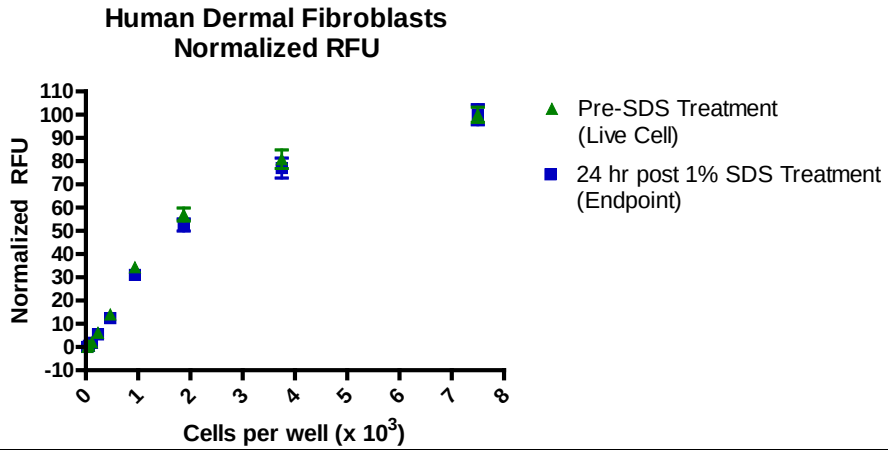


Figure 6. Using SDS to Stop and Stabilize the PrestoBlue® Reagent Reaction. Fluorescence measurements were taken for a PrestoBlue® reagent assay on Human Dermal Fibroblasts immediately following the addition of SDS to 1% final concentration, and then again 24 hours later. Nearly identical values were obtained.

What should I know before I begin setting up my assay?

Controls

- Be sure to include appropriate assay controls. We recommend the following:

  No Cell Controls - Wells that contain only culture media so that the background fluorescence can be determined and subtracted from experimental wells. We recommend using the same serum concentration in controls. Phenol red does not interfere with the assay.

  Untreated Cell Controls - If treating your cells with a compound it is recommended to plate wells of untreated cells to serve as an internal control.

Plating Density

- You may need to determine the plating density for your specific cell type when running the PrestoBlue® reagent assay to help ensure that the assay output remains within the linear range. For example, at 10 minutes Jurkat cells will show a linear correlation within a range of 100-100,000 cells/well whereas U-2OS cells have a linear range of 100-20,000. At 16 hours, Jurkat cells have a linear range of 10-10,000 cells (Figure 7).
- As shown in Figure 7, it is likely that you will see a plateau effect of fluorescent values with high numbers of cells/well. This is a result of the resazurin within the solution being reduced to resorufin which makes a secondary reduction to hydro-resorufin, a colorless, non-fluorescent compound (1).

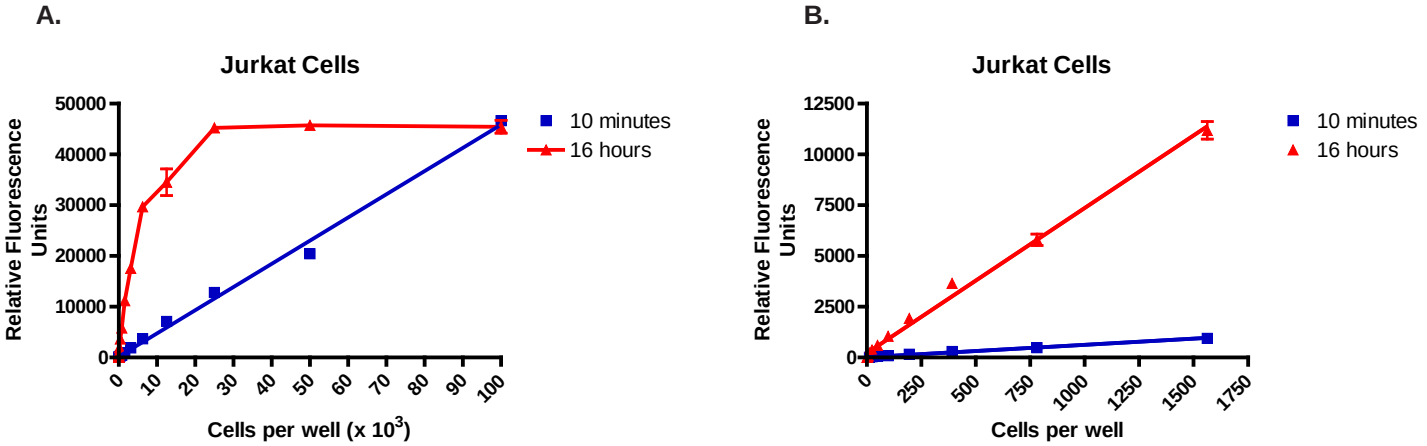


Figure 7. Effect of PrestoBlue® reagent assay incubation time on signal formation.
Serial twofold dilutions of Jurkat cells from 0–100,000 were incubated with PrestoBlue® reagent at 37°C/5%CO_2 for 10 minutes and 16 hours. The 10-minute incubation period shows a linear correlation (r2 = 0.99) between fluorescence and cell number for 0-100,000 cells (A). For the 16-hour incubation period, there is a gain in assay sensitivity but a loss of linearity above 10,000 cells/well. For a 10-minute incubation, the signal from 98 cells was greater than that from zero cells +/- 3 standard deviations. After 16 hours, the signal from 12 cells was greater than that from zero cells +/- 3 standard deviations (B).

Incubation Time

- If longer incubation times are required (overnight), be sure to maintain sterile conditions during reagent addition and incubation to help avoid microbial contamination. Contaminated cultures will yield erroneous results because microbial contaminants also reduce PrestoBlue® reagent.

- If your test compound has delayed cytotoxic effects, (i.e. cells won't show signs of distress for several hours or even days), we advise adding the PrestoBlue® reagent toward the end of the intended exposure period, after cells have been affected. If the PrestoBlue® reagent is added at the start of your experiment, viable cells will reduce resazurin before your compound can take effect, thus compromising your end results.
- If loading multiple wells of a 96 or 384-well plate using a single channel pipette, add PrestoBlue® reagent to all wells containing the same condition prior to moving to next condition for most consistent results. Begin timing after adding reagent to the last well on the plate.

II Storage and Handling

Does PrestoBlue® reagent need to be reconstituted?

No, PrestoBlue® reagent does not need to be constituted. PrestoBlue® cell viability reagent comes as a 10X, ready-to-use solution that can be added directly to the cells in culture media. Simply add 10 µL reagent to every 90 µL of sample.

How light sensitive is the PrestoBlue® cell viability reagent?

Both the resazurin dye and resorufin product are light sensitive. Storage in the provided foil bag is recommended as prolonged exposure (greater than a month) of PrestoBlue® reagent to light will increase the background fluorescence and decrease assay sensitivity. Background fluorescence can be corrected for by including no-cell control wells, but the assay window will be decreased. (Figure 7)

What if I accidentally left the PrestoBlue® reagent stock at room temperature, overnight?

You will not have any problems with PrestoBlue® reagent left at room temperature overnight. PrestoBlue® reagent is stable for up to 12 months at room temperature (~22ºC) when stored protected from light (Figure 8).

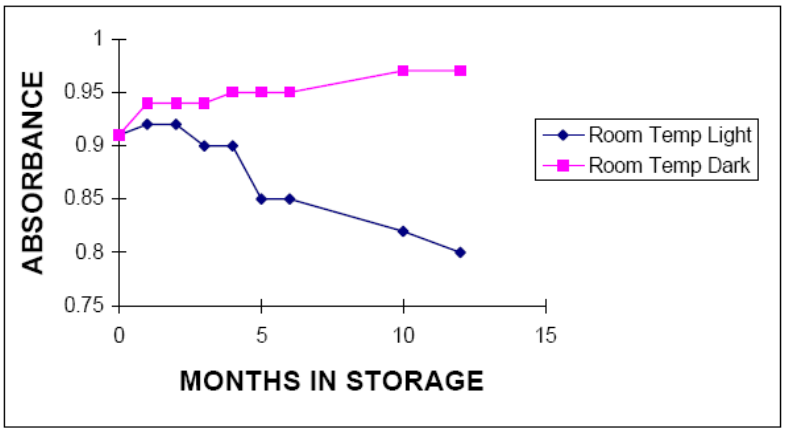


Figure 8. Effects of Light exposure and room temperature on PrestoBlue® reagent
Average absorbance at 600 nm is presented for each month tested. Light exposure was continuous at a level of approximately 100 lumens.

I accidentally froze the PrestoBlue® reagent stock, can I still use it?

You can still use PrestoBlue® reagent after it has been frozen. PrestoBlue® reagent has been tested after 5 freeze/thaw cycles with no change in assay performance. Be sure to thaw the reagent completely and mix it so that the solution is homogenous before use.

III Cell Types

Can I use PrestoBlue® reagent with suspension cells?

Yes, as shown throughout this document PrestoBlue® reagent works on adherent and suspension mammalian cells. Please see figures 1-12.

Can I use PrestoBlue® reagent on Primary Cells?

PrestoBlue® reagent has been tested on several Primary Cell lines with success (Figure 9).

A.

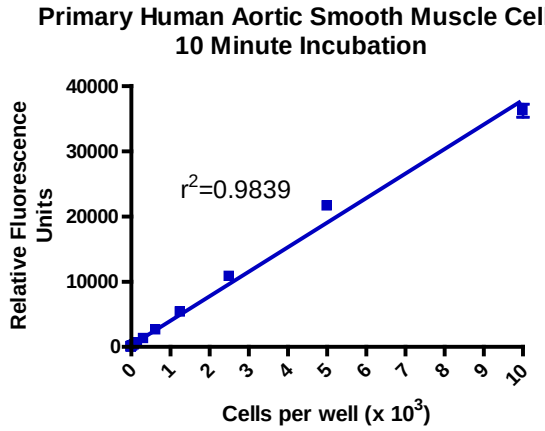
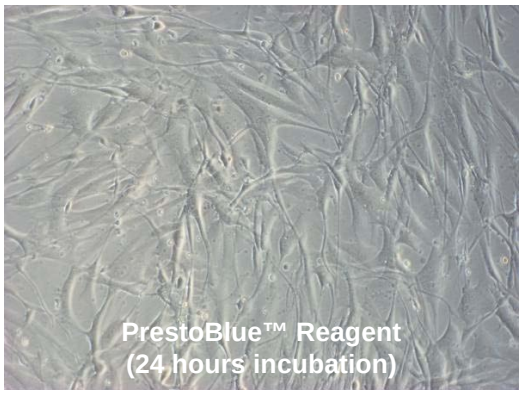


Figure 9. Use of PrestoBlue® reagent on HASMC cells.
Serial two-fold dilutions of Primary Human Aortic Smooth Muscle cells (HASMC) were prepared at 36 µl/well in a 384-well plate and cultured overnight at 37°C/5% CO_2 (A). PrestoBlue® cell viability reagent was added (4µl/well) and cells were incubated at 37°C/5% CO_2 for 10 minutes prior to recording fluorescence. HASMC cells were left untreated (B) or treated with PrestoBlue® reagent (C) for 24 hours and showed no adverse effect.

B.

C.



Can I use PrestoBlue® reagent with non-mammalian cells, such as bacteria?

PrestoBlue® cell viability reagent has been shown to work with insect and bacterial (Figure 10) cells as well as plant and fish cells (2-5).

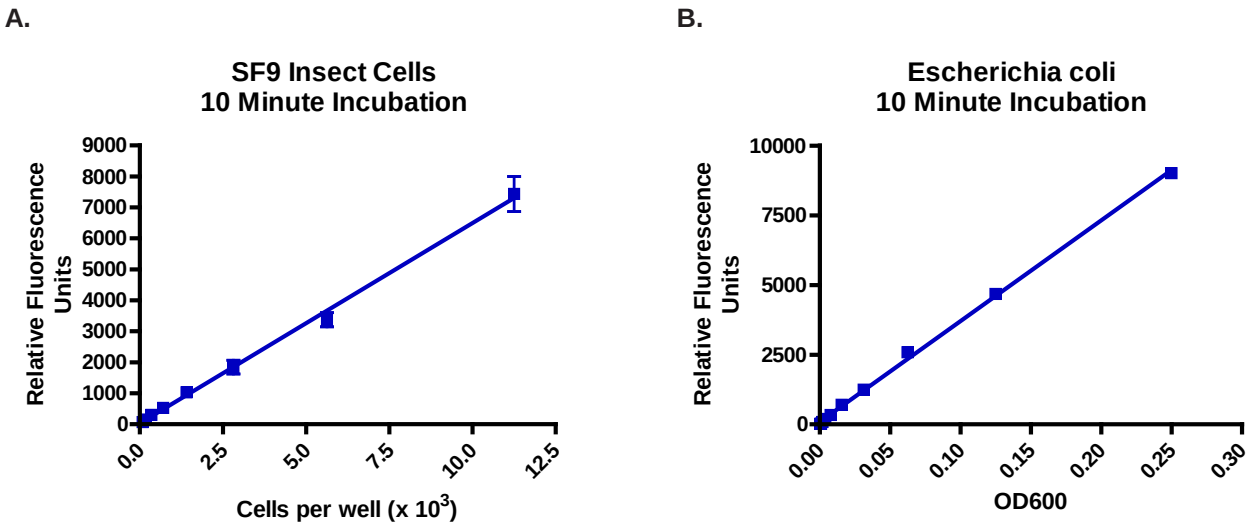


Figure 10. Use of PrestoBlue® reagent on non-mammalian cells.
Serial two-fold dilutions of SF9 (A) and Escherichia coli (B) cells were prepared at 100µl/well in a 96-well plate. PrestoBlue® reagent was immediately added (10µl/well) and cells were incubated at room temperature (A) or 37°C (B) for 10 minutes prior to recording fluorescence.

Is PrestoBlue® reagent toxic to cells?

While most reports in the literature suggest that solutions containing resazurin, the active ingredient in PrestoBlue® reagent, are not toxic to cells (6, 7), other reports clearly show that cell viability is affected depending on the length of exposure and concentration of resazurin to which they are subjected (8,9). In our experience, it is unlikely that cells will be adversely affected by exposure to PrestoBlue® reagent if assay conditions remain within the suggested incubation period (i.e. 10 minutes – 2 hours). Cells can be incubated up to 24 hours in the presence of PrestoBlue® reagent, and then further cultured. Simply remove the reagent from cells and replace it with growth medium.

IV Methods

What is the optimal incubation time? Can I incubate my cells with PrestoBlue® reagent overnight?

We recommend incubating cells with PrestoBlue® reagent for 10 minutes – 2 hours. For more sensitive detection, or when your assay contains lower numbers of cells, you may want to increase the incubation time up to 24 hours. Keep in mind that 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 (See Figure 7).

What is the optimal incubation temperature for PrestoBlue® reagent?

Cells can be incubated with PrestoBlue® reagent at either 37°C/5% CO_2 or room temperature. The PrestoBlue® reagent is more rapidly converted at 37°C, increasing the sensitivity of your assay. If incubating longer than 4 hours it is recommended to incubate at 37°C/5% CO_2 (Figure 11).

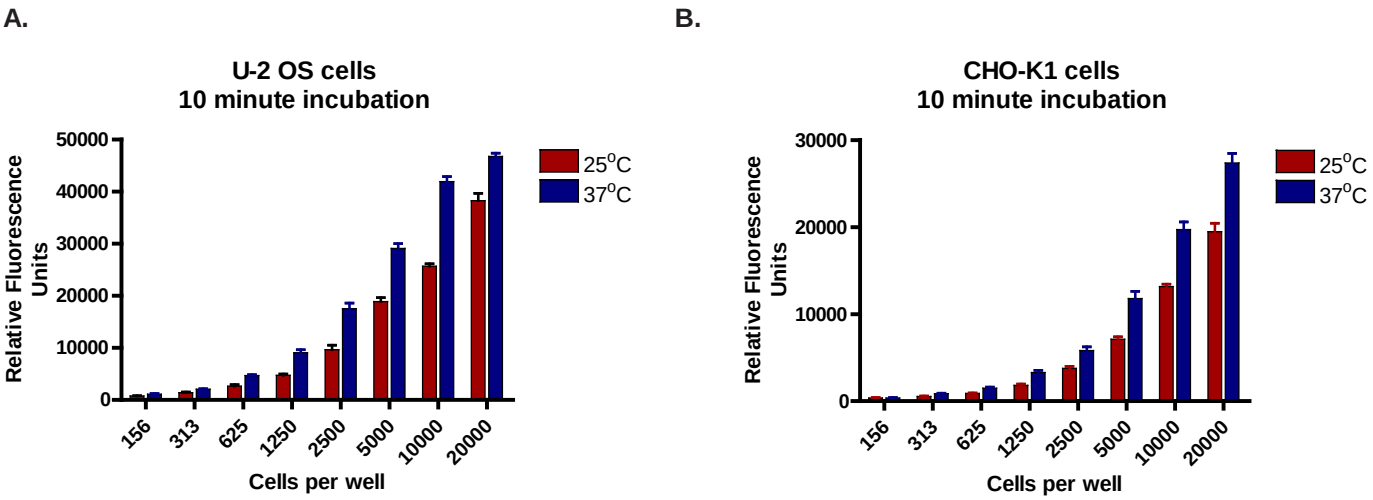


Figure 11. Performance of PrestoBlue® reagent at 37°C and 25°C.
Serial twofold dilutions of U-2 OS (A) and CHO-K1 (B) cells were incubated with PrestoBlue® reagent at either 37°C/5%CO_2 or room temperature for 10 minutes.

What if I don't have an instrument suitable for reading fluorescence?

The absorbance of PrestoBlue® 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 (Figure 12).

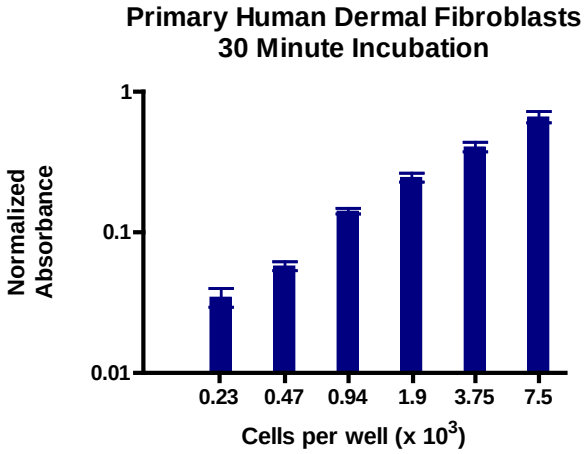


Figure 12: Absorbance measurements for Human Dermal Fibroblast cell dilutions.
Serial twofold dilutions of HDF cells were incubated at 37°C/5%CO_2 overnight and then loaded with PrestoBlue® reagent for 30 minutes. The Absorbance values were read at 570 nm and normalized to the 600 nm values. Normalized absorbance is plotted versus cell number.

Can PrestoBlue® Reagent be multiplexed with another assay?

Yes. Just be sure that the components of your secondary assay do not interfere with the fluorescent values of PrestoBlue® Reagent. For example, PrestoBlue® Reagent can be multiplexed with CyQUANT® NF (Life C35007) to assess both the metabolic activity and DNA concentration of a cell. As shown in Figure 13, there is excellent correlation of data between these two assays when assessing the cytotoxicity of Mitomycin C on HeLa cells.

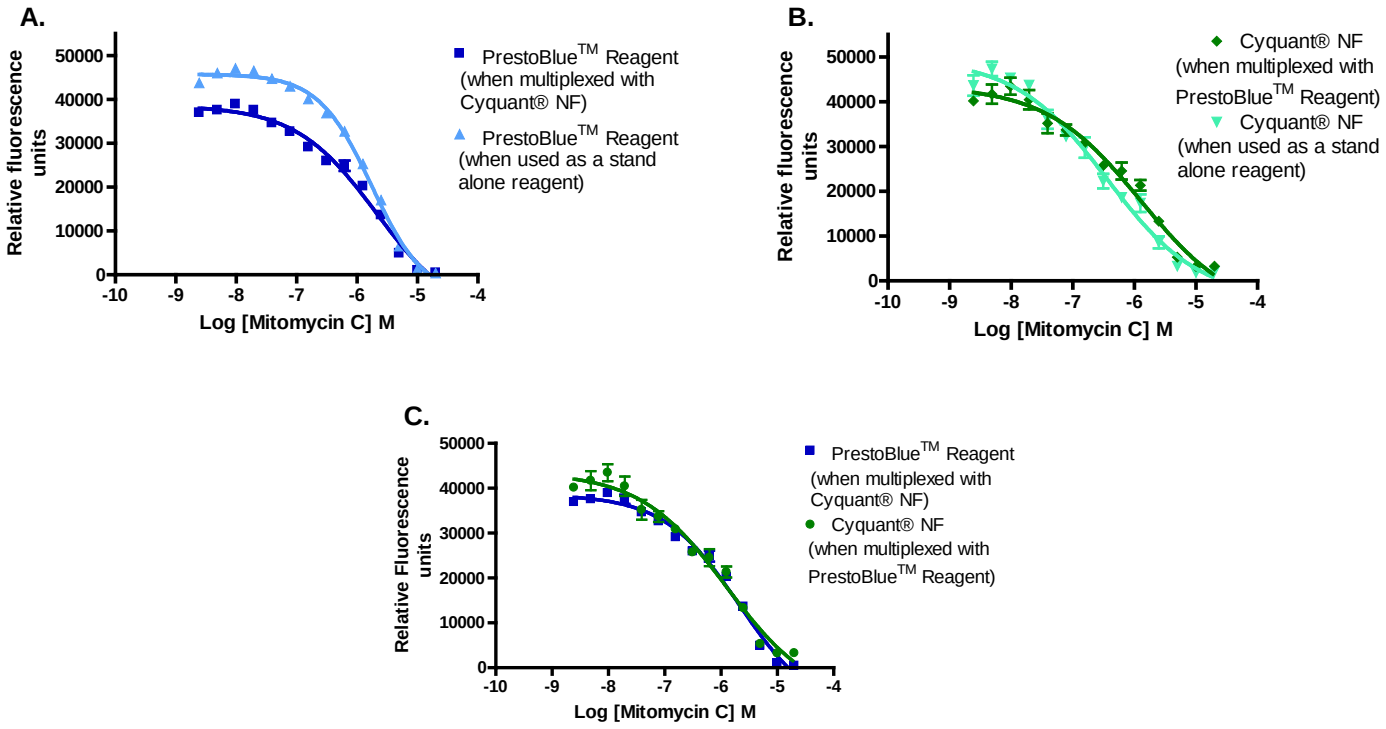


Figure 13: Multiplexing PrestoBlue® Reagent and Cyquant® NF
HeLa cells were plated in a 384 well plate at 3,000 cells per well and then exposed to various concentrations of Mitomycin C for 48 hours. The media was then removed from the cells and 1X CyQUANT® NF in HBSS, 1X PrestoBlue® Reagent in HBSS, or a combination of both reagents at 1X in HBSS was added to the cells. The cells were incubated at 37°C and 5% CO_2 for 60 minutes prior to reading out fluorescence values for all three assays. **A.** A comparison of the performance of PrestoBlue® Reagent used as a stand alone reagent or multiplexed with Cyquant® NF. **B.** A comparison of the performance of Cyquant® NF used as a stand alone reagent or multiplexed with PrestoBlue® Reagent. **C.** The performance of PrestoBlue® Reagent and Cyquant® NF when multiplexed.

V Optimizing Fluorescence Values

What could have caused high background fluorescence values in my PrestoBlue® assay?

Background fluorescence can be corrected for by including 'no cell' control wells on your assay plate and subtracting the average of those fluorescence values from your assay wells. There are a few factors that may lead to high background fluorescent values.

1. Prolonged Exposure to Light: Extended light exposure will breakdown resazurin, the active ingredient in PrestoBlue® reagent. Always store PrestoBlue® cell viability reagent in the dark and do not expose the reagent to direct light for long periods of time (see Figure 5).
2. Contamination: Bacteria will reduce resazurin to resorufin. If the reagent is compromised, it should be thrown out and replaced.

How can I raise the fluorescence intensity values for my PrestoBlue® assay?

Several factors impact the fluorescent values. To obtain higher fluorescent values try one of the following suggestions:

1. Increase the incubation time of cells with PrestoBlue® cell viability reagent.
2. Increase the number of cells plated per well.
3. Verify the instrument filter/wavelength settings.
4. Change the instrument's "gain" setting.
5. Include positive controls (living cells) in the experimental design for troubleshooting.

How can I fix high fluorescence values beyond the linear range of my?

To obtain lower fluorescent values try one of the following suggestions

1. Decrease the incubation time,
2. Reduce the number of cells plated per well.
3. Verify the instrument filter/wavelength settings.
4. Change the instrument's "gain" setting.

Help me set up my instrument!

Check your instrument's instruction manual to determine whether it is a monochrometer or filter based instrument. Then, follow the chart below. For more detailed information, contact technical support at: **probestech@invitrogen.com or phone (800) 438-2209 or (541) 335-0353**

Format	Excitation	Emission	Mirror or Dichroic
Fluorescence (Monochrometer)	560 nm 10 nm bandwidth	590 nm 10 nm bandwidth	N/A
Fluorescence (Filter)	535 25 nm bandwidth	615 nm 10 nm bandwidth	50% Mirror or 560 nm dichroic
Absorbance	570 nm	600 nm (reference wavelength for normalization)	N/A

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References

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