

reliable cell viability data  
**magical time savings**



## PrestoBlue™ Cell Viability Reagent

Reliable cell viability data in as few as 10 magical minutes

# PrestoBlue™ Cell Viability Reagent

## Why should I use the PrestoBlue™ Cell Viability Reagent?

PrestoBlue™ reagent reduces time-to-results without sacrificing performance.

- Save time with an incubation step as short as 10 minutes
- Allows for high-quality results with a large dynamic range
- Experience the convenience of a homogeneous, single addition format
- Monitor living cells and perform downstream functional assays

## How does PrestoBlue™ reagent actually work to detect cell viability?

PrestoBlue™ reagent exploits the reducing power of living cells.

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 cell proliferation, which can be used in adherent cells as well as cells in suspension. PrestoBlue™ reagent contains a cell-permeant compound that is blue in color and virtually nonfluorescent. When added to cells, PrestoBlue™ reagent is modified by the reducing environment of the viable cells, turns red in color, and becomes highly fluorescent. This change can be detected by measuring fluorescence or absorbance (Figure 1).

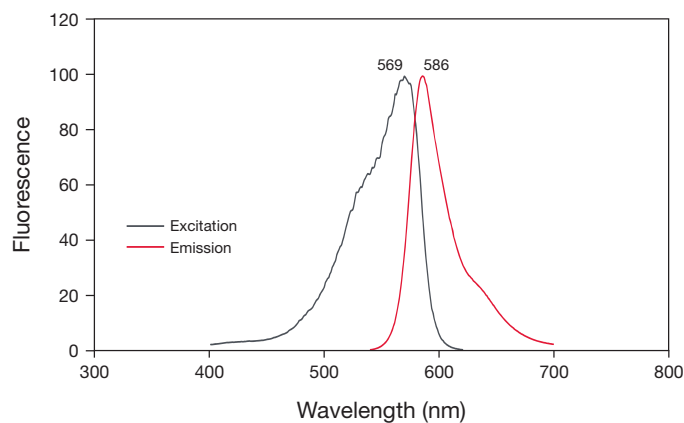
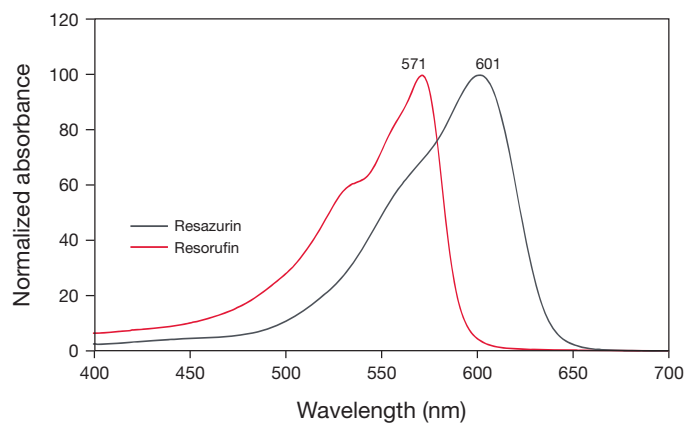


Figure 1. Absorbance and fluorescence emission spectra of PrestoBlue™ Cell Viability Reagent.

### How does the PrestoBlue™ reagent protocol save me time?

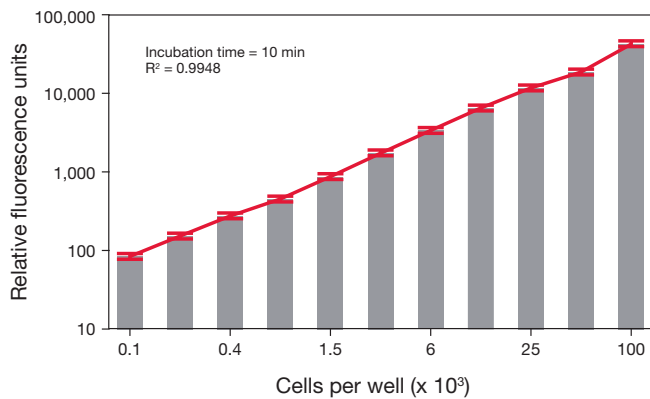
Cells only need a 10-minute incubation to deliver results.

A microplate containing the cells and the compounds to be tested is prepared. PrestoBlue™ reagent is added directly to cells and incubated at 37°C for 10 minutes. The plate is then transferred to a fluorescence reader to measure signal (Figure 3). In addition to this protocol format, other protocol considerations can be made including incubation temperature (running the assay at room temperature), incubation time, or detecting the signal using absorbance. (See the instructions in the product protocol for full details on these assay variations. The protocol can be found online at [www.invitrogen.com/prestoblue](http://www.invitrogen.com/prestoblue).)

### With such a short incubation time, what will my data look like?

You will see high-quality data in as few as 10 minutes.

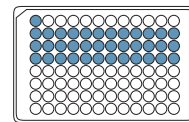
Data obtained using PrestoBlue™ reagent shows excellent linearity ( $R^2$  value of 0.9948) for cells plated at densities between 100 and 100,000 cells/well. The signal for 100 cells was greater than that from zero cells  $\pm$  3 standard deviations, and the  $Z'$  values were above 0.5 for cell densities of 391 cells/well or greater (Figure 2).



**Figure 2. Excellent data obtained in as few as 10 minutes.** Jurkat cells were plated in cell culture medium in a 384-well plate in quadruplicate starting at 100,000 cells/well with a two-fold serial dilution. Cells were incubated with PrestoBlue™ reagent for 10 minutes prior to measuring fluorescence.



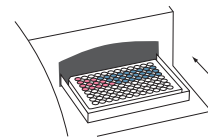
PrestoBlue™ reagent



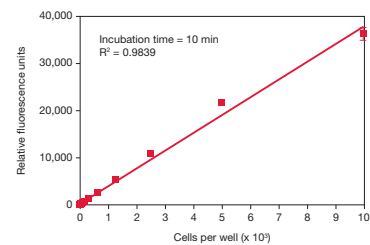
(1) Add reagent to cells



(2) Incubate



(3) Read fluorescence



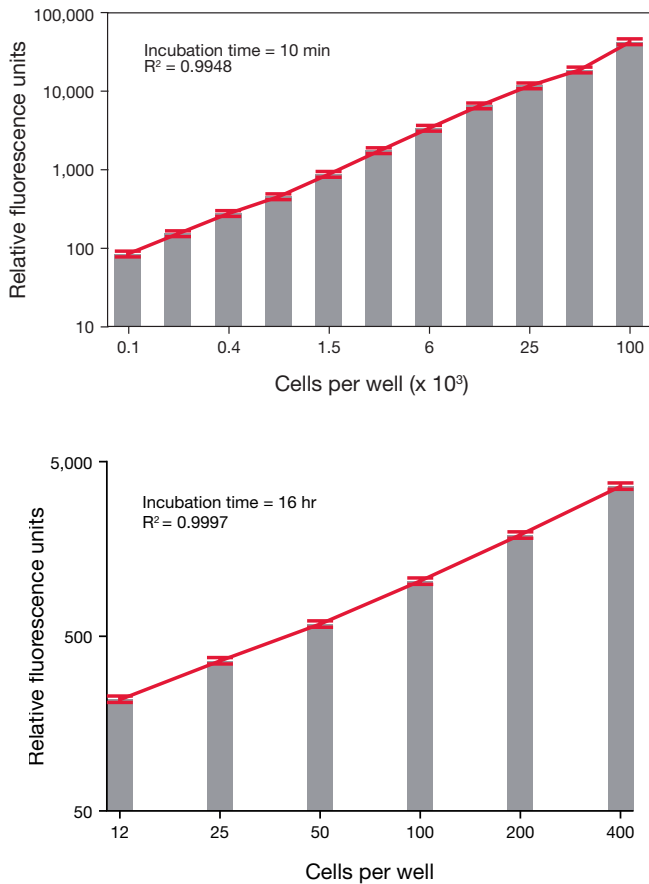
(4) Process data

**Figure 3. PrestoBlue™ Cell Viability Reagent protocol.**

### How sensitive is PrestoBlue™ reagent?

Depending on the cell type and incubation time, PrestoBlue™ reagent can detect as few as 12 cells per well.

At 10 minutes of incubation, PrestoBlue™ reagent can detect as few as 98 cells/well, and the signal remains linear over the a broad dilution range. Following a 16-hour incubation with PrestoBlue™ reagent, the signal for 12 cells was greater than that from zero cells  $\pm$  3 standard deviations, and the Z' values were above 0.5 for cell densities of 24 cells/well or greater (Figure 4). Note that at low cell densities, the signal-to-background ratio increases after 16 hours of incubation.

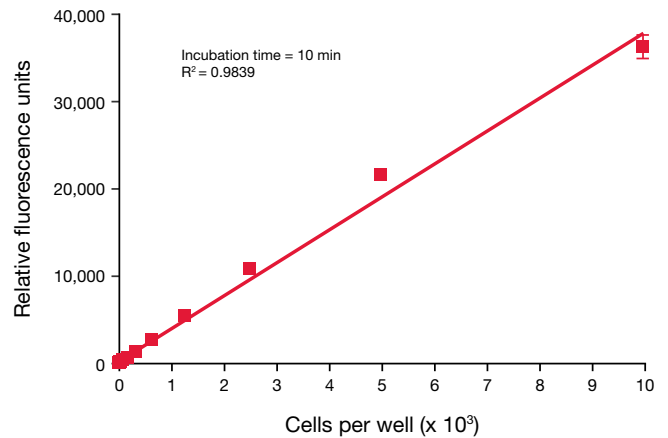


**Figure 4. Extremely sensitive detection.** Jurkat cells were plated in cell culture medium in a 384-well plate in quadruplicate starting at 100,000 cells/well with a two-fold serial dilution. Fluorescence measurements were taken following 10 minutes of incubation and following 16 hours of incubation with PrestoBlue™ reagent.

### Can I use PrestoBlue™ reagent with primary cells?

Yes, PrestoBlue™ reagent is compatible with primary cells.

PrestoBlue™ reagent has been tested with but is not limited to the following cell types: human dermal fibroblasts, human aortic smooth muscle cells, and human epidermal keratinocytes, neonatal. Cells remain viable, so you can perform downstream functional assays with your cells after you have assessed their viability status. PrestoBlue™ reagent is the ideal option when using cells available in a limited supply such as primary cells (Figure 5).

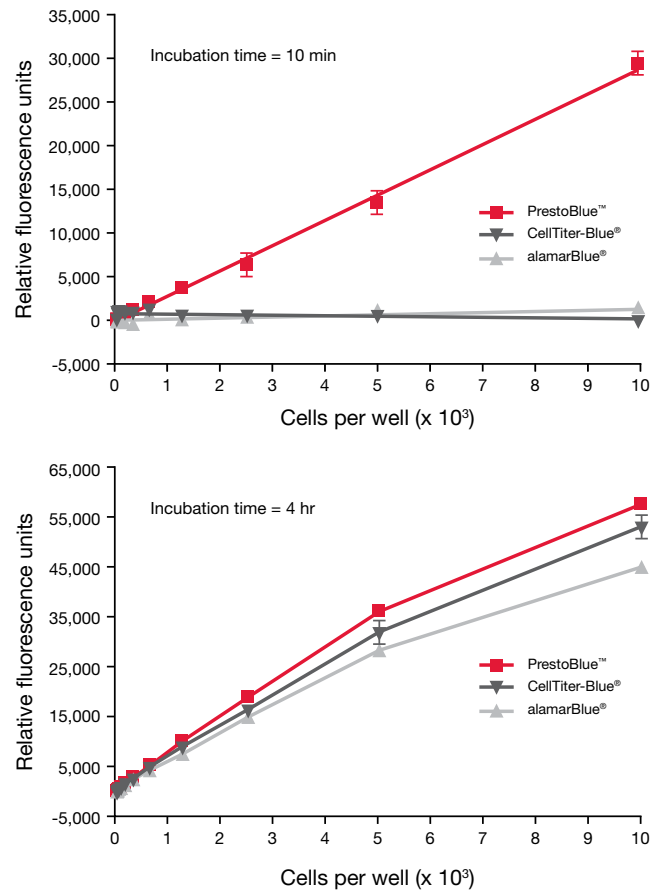


**Figure 5. Cell viability results in primary cells.** Human aortic smooth muscle cells were plated in cell culture medium in a 384-well plate in quadruplicate starting at 100,000 cells/well with a two-fold serial dilution. Fluorescence measurements were taken following 10 minutes of incubation with PrestoBlue™ reagent.

### Why choose PrestoBlue™ reagent over alamarBlue®, CellTiter-Blue®, or other rezasurin-based reagents?

Viability measurements using PrestoBlue™ reagent are significantly faster and deliver comparable results.

PrestoBlue™ reagent only requires a 10-minute incubation period, whereas alamarBlue® and CellTiter-Blue® reagent protocols recommend incubation times from 1 to 4 hours. Figure 6 demonstrates the difference in fluorescence signal obtained from PrestoBlue™ reagent and CellTiter-Blue® reagent following a 10-minute incubation. The dynamic range is significantly greater at the early time points for PrestoBlue™ reagent.



**Figure 6. Achieve assay results with PrestoBlue™ reagent in a fraction of the time.** CHO-K1 cells were plated in cell culture medium in a 384-well plate in quadruplicate starting at 10,000 cells/well with a two-fold serial dilution. Cells were incubated with PrestoBlue™ reagent, alamarBlue® reagent, or CellTiter-Blue® reagent for 10 minutes or 4 hours. Fluorescence measurements obtained with PrestoBlue™ reagent in 10 minutes take 4 hours with alamarBlue® and CellTiter-Blue® reagents.

### What advantages does PrestoBlue™ reagent have over tetrazolium salts such as MTT?

There are many reasons why PrestoBlue™ reagent is preferable to MTT and XTT.

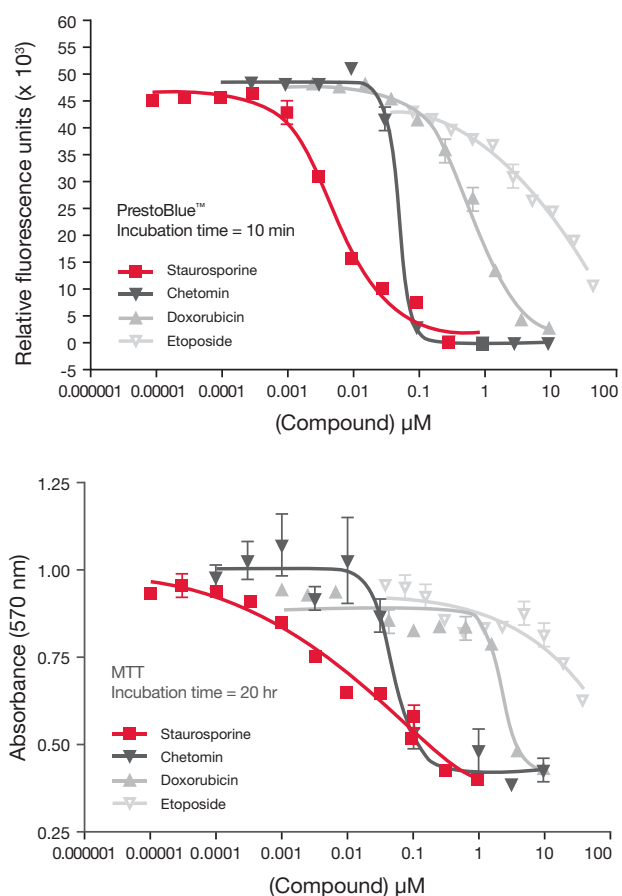
PrestoBlue™ reagent is the ideal choice if you want:

- Comparable data with faster time-to-results and fewer protocol steps (Table 1 and Figure 7)
- To keep your cells alive for downstream assays
- A safer reagent that minimizes toxic waste considerations
- A ready-to-use aqueous solution

Switch from MTT to PrestoBlue™ reagent to simplify your cell viability assays.

**Table 1. Reduced time-to-results with PrestoBlue™ reagent.** With PrestoBlue™ reagent, results can be obtained in as few as 10 minutes as compared to a minimum of 1 hour for XTT, or 2 hours for MTT.

	MTT	XTT	PrestoBlue™
Minimum incubation time	1 hr	1 hr	10 min
Solubilization time	1 hr	N/A	N/A
Total time	2 hr	1 hr	10 min



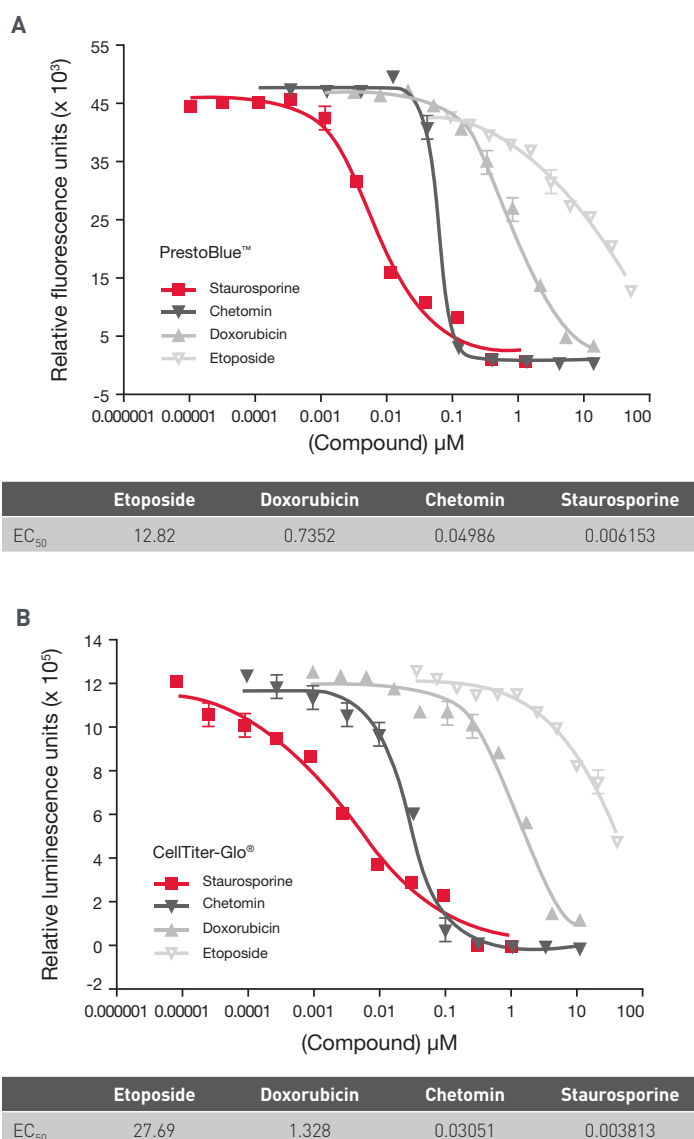
	PrestoBlue™ Reagent EC <sub>50</sub> (μM)	MTT EC <sub>50</sub> (μM)
Etoposide	12.82	220.9
Doxorubicin	0.7352	2.29
Chetomin	0.04986	0.05302
Staurosporine	0.005153	0.03626

**Figure 7. Comparison of PrestoBlue™ reagent to MTT.** Comparable rank order potency results were obtained with PrestoBlue™ reagent and MTT. U2OS 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<sub>50</sub> values were obtained for each compound.

### How does PrestoBlue™ reagent compare to CellTiter-Glo® reagent?

**PrestoBlue™ reagent allows for continuous live-cell monitoring and assessment after measuring viability.**

PrestoBlue™ reagent does not require cell lysis, in contrast to CellTiter-Glo® reagent, allowing you to leave your cell samples in PrestoBlue™ reagent and read the signal up to 24 hours later. With PrestoBlue™ reagent, cells remain viable, so you can perform downstream functional assays with your cells after you have assessed their viability status. If you are using cells that are in limited supply such as primary cells, PrestoBlue™ reagent is an excellent choice.



**Figure 8. Comparable results obtained with PrestoBlue™ reagent or CellTiter-Glo® reagent in 10 minutes.** U2OS 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 incubated for 10 minutes with (A) PrestoBlue™ reagent or (B) CellTiter-Glo® reagent prior to measuring fluorescence (PrestoBlue™ reagent) or luminescence (CellTiter-Glo® reagent). Comparable EC<sub>50</sub> values were obtained for each compound.

### What are the incubation conditions of PrestoBlue™ reagent? Can I incubate longer than 10 minutes?

Cells assessed using PrestoBlue™ reagent can be incubated for longer than 10 minutes.

We recommend incubating cells with PrestoBlue™ reagent for 10 minutes to 2 hours. For more sensitive detection, or when your assay contains fewer cells, you may increase the incubation time up to 24 hours. Keep in mind that signals from samples of higher cell density may be “saturated,” which means the linearity of detection may have reached a plateau. If this occurs, we recommend decreasing the incubation time.

Cells can be incubated with PrestoBlue™ reagent at either 37°C/5% CO<sub>2</sub> or room temperature. PrestoBlue™ reagent is more rapidly converted at 37°C, increasing the sensitivity of your assay. If incubating longer than 4 hours, we recommend incubation at 37°C/5% CO<sub>2</sub>.

Because PrestoBlue™ reagent is a live-cell assay, readings may be taken at multiple time points to determine the optimal performance in your lab.

### How does my instrument need to be set up for PrestoBlue™ reagent?

Readouts for PrestoBlue™ reagent can be performed using instruments that measure fluorescence (top or bottom read) or absorbance.

Recommended incubation times vary depending on the type of instrument and readout you are using. See Tables 2 and 3 for instrument setup recommendations.

For questions regarding instrument setup, contact our Technical Support team at [probestech@invitrogen.com](mailto:probestech@invitrogen.com).

### Who do I contact for technical questions?

Our Technical Support teams are here to help you.

Email us: [probestech@lifetech.com](mailto:probestech@lifetech.com)

Call us: **North America:** 800 438 2209

**International:** 760 603 7200

### Where can I get more information and the detailed protocol for PrestoBlue™ reagent?

Visit our website at [www.invitrogen.com/prestoblue](http://www.invitrogen.com/prestoblue) for more product information, data, protocols, and troubleshooting tips.

Table 2. Recommended incubation times for PrestoBlue™ reagent.

Format	Recommended incubation time
Bottom-read fluorescence	10 minutes – 2 hours
Top-read fluorescence	30 minutes – 2 hours
Absorbance	20 minutes – 2 hours
Room-temperature incubation	10 minutes – 2 hours
Low cell number (<5,000 cells/100 µL)	20 minutes – 24 hours

Table 3. Recommended excitation and emission spectra for PrestoBlue™ reagent.

Format	Excitation	Emission
General	540–570 nm	580–610 nm
Fluorescence (Monochromator)	560 nm (10 nm bandwidth)	590 nm (10 nm bandwidth)
Fluorescence (Filter)	535 nm (10 nm bandwidth)	615 nm (10 nm bandwidth)
Absorbance	570 nm	600 nm (reference wavelength for normalization)

### Ordering information

Product	Qty	Cat. No.
PrestoBlue™ Cell Viability Reagent	25 mL	A13261
PrestoBlue™ Cell Viability Reagent	100 mL	A13262

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