The PrestoBlue™ Cell Viability Reagent: Reliable Cell Viability Data in as Few as 10 Minutes

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INTRODUCTION

The PrestoBlue™ Cell Viability Reagent is a resazurin-based solution that uses the reducing ability of living cells to quantitatively measure cell proliferation. When cells are alive and healthy, they maintain a reducing environment within their cytosol. Upon entering a living cell, PrestoBlue™ reagent is reduced to resorufin which is red in color and highly fluorescent. The health of the cell can be monitored by the change in fluorescence and/or absorbance. Metabolically active cells continuously convert the PrestoBlue™ reagent. Non-viable cells cannot reduce the indicator dye and therefore do not generate a change in signal.

Whereas other commercially available resazurin solutions (such as CellTiter-Blue® or alamarBlue® Reagents) require a 1-4 hour incubation in order to generate a significant signal, we show that PrestoBlue™ reagent generates a linear signal with excellent sensitivity in response to cell number in as few as 10 minutes. We also show that the reagent can be used with a wide variety of mammalian and non-mammalian cell types including primary cells, stem cells, insect cells, and bacterial cells. In addition, we show that the IC_{50} values generated from cytotoxic agents is similar for PrestoBlue™ reagent as other common cell viability reagents like CellTiter-Glo® and MTT.

RESULTS

Figure 1. PrestoBlue™ Cell Viability Reagent Workflow

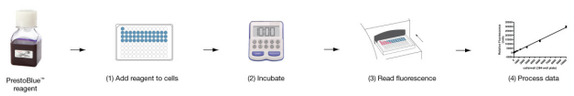


Figure 21– Workflow of the PrestoBlue™ Cell Viability Reagent. The PrestoBlue™ Cell Viability Reagent comes as a ready to use 10X Solution. Simply add the PrestoBlue™ reagent to cells, incubate for as little as 10 minutes, read the fluorescence (or absorbance), and process the data.

PrestoBlue™ Cell Viability Reagent can be used with cells plated in their standard growth media containing serum and phenol red. For absorbance reads, it is recommended that cells be incubated at least 20 minutes with the PrestoBlue™ reagent.

Figure 2A. Absorbance Spectra

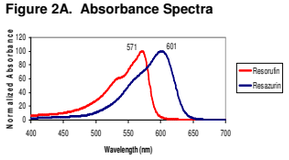


Figure 2B. Fluorescence Spectra of Resorufin

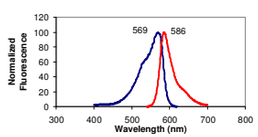


Figure 2C. Image of PrestoBlue™ Reagent At Work

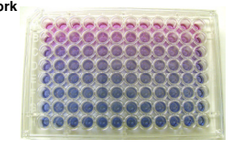


Figure 2. Spectral Properties of the PrestoBlue™ Cell Viability Reagent. PrestoBlue™ Cell Viability Reagent is a resazurin-based solution. Resazurin has a maximum absorbance of ~600 nm, appears blue in color, and is non-fluorescent. Upon reduction, resazurin is converted to resorufin. Resorufin has a maximum absorbance of ~570 nm, appears red in color, and is highly fluorescent. When PrestoBlue™ reagent is added to cells, metabolically active cells will convert the resazurin to resorufin, allowing the relative number of metabolically active cells in a well to be determined by absorbance or fluorescence.

2A) The normalized absorbance spectra of resazurin and resorufin.

2B) The normalized fluorescence excitation and emission spectra of resorufin.

2C) An image of a 96 well plate containing a dilution series of CHO-K1 cells loaded with PrestoBlue™ reagent. The bottom wells contain no cells. As resazurin is converted to resorufin by metabolically active cells, the wells appear more pink in color.

Figure 3. PrestoBlue™ Reagent Performance Compared to alamarBlu® and CellTiter-Blue®

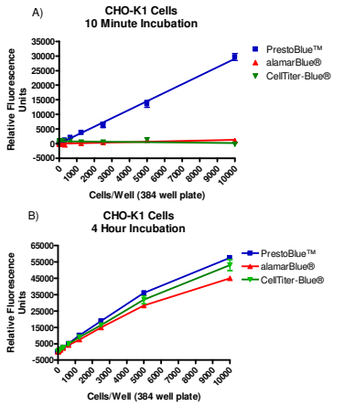


Figure 3. The PrestoBlue™ Cell Viability Reagent Works in Just 10 minutes. CHO-K1 cells were plated in complete growth medium in a 2 fold dilution series in a 384 well plate starting at 10,000 cells/well. Cells were incubated overnight at 37°C/5% CO_2. The following day, cells were loaded with PrestoBlue™, alamarBlue®, or CellTiter-Blue® reagent according to manufacturers' directions. Cells were then incubated at 37°C/5% CO_2 for either 10 minutes or 4 hours prior to reading out fluorescence. A) PrestoBlue™ exhibits a linear signal in just 10 minutes, whereas the other resazurin based products are inactive. B) At 4 hours (a 1-4 incubation time is recommended for other resazurin products), all three reagents show similar results.

Figure 4. Linearity and Sensitivity of PrestoBlue™ Cell Viability Reagent

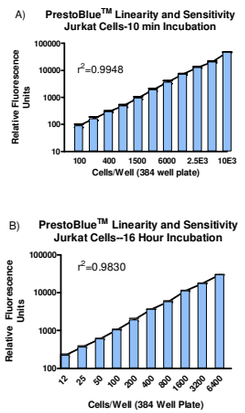


Figure 4. PrestoBlue™ Cell Viability Reagent Shows Excellent Linearity and Sensitivity. Jurkat cells were in complete growth medium in a 2 fold dilution series in a 384 well plate starting at 100,000 cells/well. Cells were immediately loaded with PretoBlue™ Cell Viability Reagent and incubated for 10 minutes or overnight (16 hours) prior to reading out fluorescence. In just 10 minutes, the PrestoBlue™ reagent shows linearity (r^2=0.9948) between 100-100,000 cells. When Incubated overnight, PrestoBlue™ reagent shows linearity between 10-6400 cells/well.

Figure 5. PrestoBlue™ Cell Viability Reagent Can Be Multiplexed with Other Assays

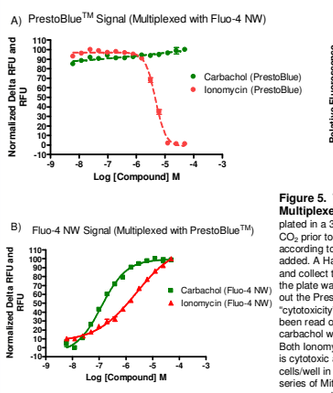
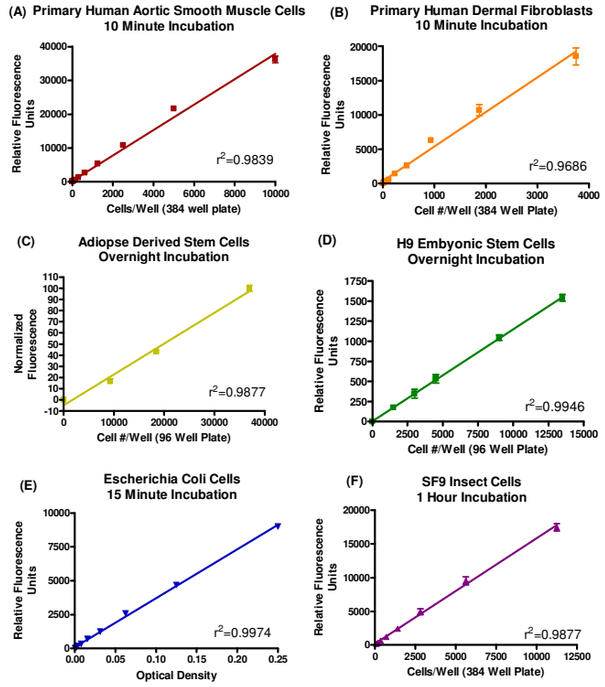


Figure 5. The PrestoBlue™ Cell Viability Reagent Can Be Multiplexed with Other Assays. A) and B) CHO-k1 cells were plated in a 384 well plate at 10,000 cells/well and incubated at 37°C/5% CO_2 prior to running the assay. Cells were then loaded with Fluo-4 NW according to the manufacturer's protocol with PrestoBlue™ reagent added. A Hamamatsu FDSS was used to add compounds to the plate and collect the Fluo-4 signal. Following collection of the Fluo-4 signal, the plate was returned to the incubator for 3 ½ hours prior to reading out the PrestoBlue™ reagent signal. (Note: This allowed extra time for "cytotoxicity". Alternatively, the PrestoBlue™ reagent signal could have been read out immediately. A) The PrestoBlue™ reagent shows that carbachol was not cytotoxic to the cells whereas ionomycin was. B) Both Ionomycin and Carbachol show calcium responses but Ionomycin is cytotoxic as shown in A). C) HeLa cells were plated at 2500 cells/well in a 384 well plate and incubated for 48 hours with a diltuion series of Mitomycin C. Media was removed from the well (as recommended by the Cyquant® protocol), and replaced with HBSS containing 1X PrestoBlue™ reagent and 1X Cyquant® reagent. A similar cytotoxicity profile is obtained using these two viability assays multiplexed.

Figure 6. PrestoBlue™ Cell Viability Reagent Works on a Variety of Cell Types and is a Live Cell Assay Allowing Results to be Obtained at Various Time Points



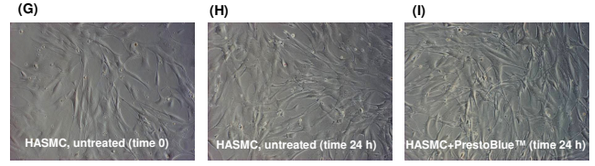
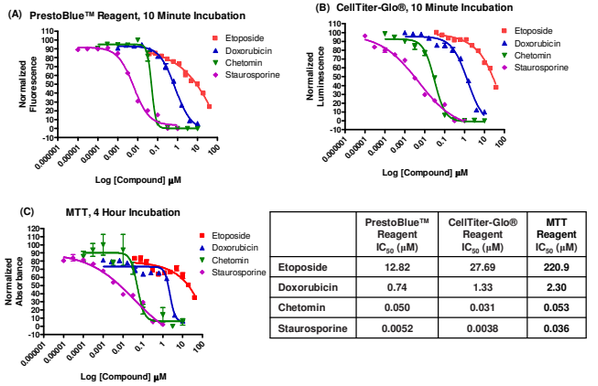


Figure 6. The PrestoBlue™ Cell Viability Reagent Can Be Used With a Variety of Cell Types. (A), (B), (C), (D), (E), and (F) Cell types as indicated were plated at the indicated cell densities and incubated for the indicated time with PrestoBlue™ reagent prior to reading out the fluorescence. All cell types tested including primary cells, stem cells, bacterial cells, and insect cells exhibited a linear correlation between cell number and fluorescent signal. The PrestoBlue™ Cell Viability Reagent is a live cell readout, so multiple read-outs can be taken throughout the day. Primary human aortic smooth muscle cells were left untreated (G) and (H) or were treated (I) with PrestoBlue™ Reagent for 24 hours. Images show that the cells in the presence of PrestoBlue™ reagent for 24 hours remain well attached to the plate and were able to divide with no overt toxicity.

Figure 8. Similar IC_{50} Values of Cytotoxic Compounds are Obtained with PrestoBlue™ Cell Viability Reagent, CellTiter-Glo®, and MTT.



	PrestoBlue™ Reagent IC_{50} (µM)	CellTiter-Glo® Reagent IC_{50} (µM)	MTT Reagent IC_{50} (µM)
Etoposide	12.82	27.69	220.9
Doxorubicin	0.74	1.33	2.30
Chetomin	0.050	0.031	0.053
Staurosporine	0.0052	0.0038	0.036

Figure 8. Similar IC_{50} Values of Cytotoxic Compounds are Obtained with PrestoBlue™ Cell Viability Reagent, CellTiter-Glo®, and MTT. U-2 OS 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 (A) 10 minutes with PrestoBlue™ reagent, (B) 10 minutes with CellTiter-Glo® reagent, or (C) 4 hours with MTT (followed by a solubilization step) prior to reading out the fluorescence (PrestoBlue™ reagent), luminescence (CellTiter-Glo® reagent), or absorbance (MTT reagent) values. The same rank order potency of the compounds and comparable IC_{50} values were obtained for the three reagents. Both the MTT and CellTiter-Glo® reagents required cell lysis whereas the PrestoBlue™ reagent is a live cell assay.

CONCLUSIONS

- The PrestoBlue™ Cell Viability Reagent leads to reliable cell viability data in as little as 10 minutes whereas other resazurin-based assays are inactive in 10 minutes.
- The PrestoBlue™ Cell Viability Reagent is extremely sensitive allowing 100 cells/well to be detected in just 10 minutes or 10 cells/well to be detected with a longer incubation period.
- The PrestoBlue™ Cell Viability Reagent is a live cell assay making multiplexing with other assay readouts straightforward.
- The PrestoBlue™ Cell Viability Reagent is compatible with a wide variety of cell types including primary cells, stem cells, bacterial cells, and insect cells.
- The PrestoBlue™ Cell Viability Reagent produces accurate IC_{50} values for cytotoxic compounds and does not require cell lysis as other common cell viability reagents (like CellTiter-Glo® and MTT) do.
- For more information or to order PrestoBlue™ go to: www.invitrogen.com/prestoblue

TRADEMARKS