invitrogen PRODUCT INFORMATION SHEET

PrestoBlue™ HS Cell Viability Reagent

Catalog Numbers P50200 and P50201

Pub. No. MAN0018371 **Rev.** B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Contents and storage

Sufficient material is supplied for 1,000 × 96-well assays (Cat. No. P50200) or 10,000 × 96-well assays (Cat. No. P50201) based on the protocol that is described in this document.

Component	Cat. No. P50200	Cat. No. P50201	Storage[1]
PrestoBlue™ HS Cell Viability Reagent	1 × 25 mL (10X)	1 × 100 mL (10X)	• Store at 2–8°C. • Protect from light.

[1] When stored as directed, the kit is stable for 6 months from date of receipt.

- Fluorescence excitation and emission ranges: 540–570 nm and 580–610 nm.
  Fluorescence excitation/emission maxima: 560/590 nm
- Absorbance: Monitor the absorbance of the cell viability reagent at 570 nm, using 600 nm as a reference wavelength (normalized to the 600-nm value).

Product description

Measuring changes in cell viability is a fundamental method for evaluating cell health, determining genotoxicity, and evaluating anti-cancer drugs. Cell health can be monitored by detecting changes in several key indicators. These include changes to plasma membrane integrity, DNA synthesis, DNA content, enzyme activity, presence of ATP, and cellular reducing environment.

Monitoring changes to the cellular reducing environment or metabolic activity by using resazurin-based reagents is a well-established and reliable indicator of cell viability or death. On entering live cells, the cellular reducing environment reduces resazurin to resorufin, a compound that is red and highly fluorescent. Viable cells continuously convert resazurin to resorufin, increasing the overall fluorescence and color of the media surrounding the cells. Also, the conversion of resazurin to resorufin results in a pronounced color change, therefore cell viability can be detected using absorbance-based plate readers.

As a consequence of the synthesis and manufacturing processes, all resazurin-based reagents contain a detectable amount of highly fluorescent resorufin contamination. The amount of the resorufin contamination can vary greatly between sources of the material and manufacturing conditions. The varying amounts of resorufin contribute to differences in detectable background fluorescence. More importantly, the contaminating resorufin and the resulting higher background signal significantly reduces the signal to background ratio and dynamic range of the assay.

To improve the performance of the resazurin-based reagents, an innovative process was developed and on implementation removes the contaminating resorufin resulting in highly purified resazurin. To create PrestoBlue™ HS Cell Viability Reagent, the highly purified resazurin has been formulated with a proprietary buffering system.

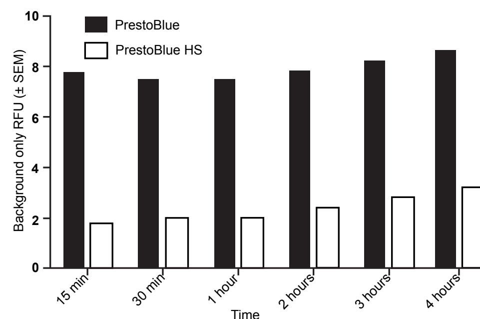

Figure 1 Reduction of background fluorescence displayed with PrestoBlue™ HS reagent compared to PrestoBlue™ reagent

100 µL of complete growth media was added to several wells of a 96-well plate. 10 µL of either PrestoBlue™ HS or PrestoBlue™ reagent was added to the wells containing complete growth media and the fluorescence was detected at various times post-addition. The PrestoBlue™ HS consistently displays a >50% reduction in background fluorescence.

On comparison, PrestoBlue™ HS reagent displays a significant reduction in background fluorescence (Figure 1) and a greatly increased signal to background ratio (Figure 2).

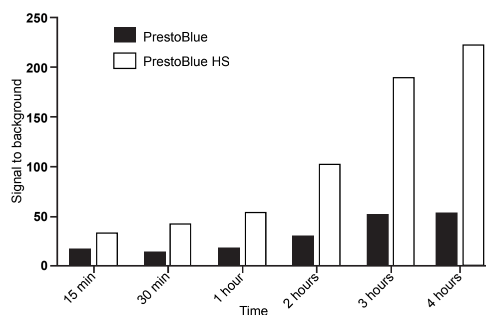

Figure 2 PrestoBlue™ HS displays a significant increase in the signal to background ratio

A549 cells were seeded at 5,000 cell/well and incubated overnight to allow for attachment. 10 µL of PrestoBlue™ HS or PrestoBlue™ reagent was added to the wells containing 100 µL of complete growth media and the attached cells. At various time points post-addition the fluorescence was measured. The results show the >100% increase in the signal to background ratio for the PrestoBlue™ HS versus the PrestoBlue™ reagent.

Unlike other resazurin-based reagents, the PrestoBlue™ HS Cell Viability Reagent has been formulated with a proprietary buffering system that resulted in a reagent with a physiological pH range optimal for the fast determination of mammalian cell viability. Cell viability can be detected with a short 10-minute incubation with the PrestoBlue™ HS Cell Viability Reagent.

The PrestoBlue™ HS Cell Viability Reagent is a complete add and read, nontoxic reagent that does not require cell lysis. The highly purified resazurin that is used for PrestoBlue™ HS results in a reagent with a >50% decrease in background fluorescence and a >100% increase in the signal to background ratio. Since no lysis is required the diluted PrestoBlue™ HS solution can be removed, then replaced with complete growth media and the cells cultured further.

Seed and treat cells

1. One day before your experiment, seed cells into a 96-well plate containing 100 µL/well of cell culture medium.

   Note: If you are using a 384-well plate, use 50 µL/well of cell culture medium.

2. Incubate the cells overnight in a 37°C incubator using the appropriate CO_2 percentage.

3. (*Optional*) Treat cells with the test compound 24–72 hours before performing the viability with the cell viability reagent.

Determine cell viability

1. Warm the cell viability reagent to room temperature before use.

2. Add 1/10th volume of cell viability reagent directly to cells in culture medium according to the following table.

Format	Volume of cells + medium	Volume of 10X cell viability reagent to add
Cuvette	900 µL	100 µL
96-well plate	90 µL	10 µL
384-well plate	36 µL	4 µL

3. Incubate for ≥10 minutes at 37°C in a cell culture incubator, **protected from direct light**.

   Note: Longer incubation times increase the sensitivity of detection. We recommend using up to a 3-hour incubation time to increase sensitivity. As this is a live cell assay, readings can be taken at multiple time points.

4. Record results using the following fluorescence or absorbance values:
   - **Fluorescence:** Read fluorescence using a fluorescence excitation wavelength of 560 nm (excitation range is 540–570 nm) and an emission of 590 nm (emission range is 580–610–nm).
   - **Absorbance:** Monitor the absorbance of reagent at 570 nm, using 600 nm as a reference wavelength (normalized to the 600-nm value).

   Note: Assay plates or tubes can be wrapped in foil, stored at 4°C, then read in 1–3 days without affecting the fluorescence or absorbance values.

5. (*Optional*) Add 50 µL of 3% SDS directly to 100 µL of cells in cell viability reagent to stop the reaction.

Limited product warranty

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