# PrestoBlue™ Cell Viability Reagent

**Catalog Numbers** A13261 and A13262

**Pub. No.** MAN0018370 **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. A13261) or 10,000 × 96-well assays (Cat. No. A13262) based on the protocol that is described in this document.

| Component | Cat. No. A13261 | Cat. No. A13262 | Storage[1] |
|---|---|---|---|
| PrestoBlue™ Cell Viability Reagent | 1 × 25 mL (10X) | 1 × 100 mL (10X) | • Store at 2–8°C.<br>• 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 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.

Resazurin, the active ingredient of PrestoBlue™ Cell Viability Reagent, is a nontoxic, cell-permeable compound that is blue in color and virtually non-fluorescent. 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.

Unlike other resazurin-based reagents, PrestoBlue™ 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. Incubation times using PrestoBlue™ are shorter than with other resazurin-based cell viability reagents. Cell viability can be detected with a short 10-minute incubation with the PrestoBlue™ Cell Viability Reagent.

The PrestoBlue™ Cell Viability Reagent is a complete add and read, nontoxic reagent that does not require cell lysis. Since no lysis is required the diluted PrestoBlue™ solution can be removed, then replaced with complete growth media and the cells cultured further.

Based on the cell type and incubation time, PrestoBlue™ Cell Viability Reagent can detect as few as 12 cells per well in a 384-well plate. After ten minutes of incubation, the PrestoBlue™ reagent can linearly detect cells down to 98 cells/well with a Z' value of greater than 0.5.

## 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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