

**Cell Meter™ RX660 fixable viability dye**

 Catalog number: 22530  
 Unit size: 200 Tests

Component	Storage	Amount
Cell Meter™ RX660 fixable viability dye	Freeze (< -15 °C), Minimize light exposure	200 tests

**OVERVIEW**

Discrimination and exclusion of dead cells from live cells allows cleaner separation and identification of cell populations. Cell Meter™ fixable viability dyes are a large family of cell-impermeable fluorescent viability dyes that are optimized to match the major excitation lasers of common flow cytometers, such as 350, 405, 488, 633 and 647 nm. These dyes are impermeant to live cells but permeant to cells with compromised membranes. They irreversibly react with amine- and thiol-containing proteins and other cellular components. Since dead or fixed cells with a compromised membrane more readily react with Cell Meter™ fixable cell stains, thus stain brighter than live cells with an intact membrane, these dyes can be used to assess live vs. dead status of mammalian cells. There are a few factors to be considered when using these dyes, e.g., the titration of each dye to ensure that live cells have minimal to no staining. Cell Meter™ RX660 fixable cell stain is optimized to be excited with the red lasers at 633/647 nm with emission at 660 nm. Compared to other commercially similar viability dyes, this fixable viability dye is much more robust and stable.

**AT A GLANCE**
**Protocol summary**

1. Prepare samples in HHBS (0.5 mL/assay)
2. Add Cell Meter™ RX660 to the cell suspension
3. Stain the cells at room temperature for 20 - 60 minutes
4. Wash the cells
5. Fix the cells (optional)
6. Examine the sample with flow cytometer using 660/20 nm filter (APC channel) and/or fluorescence microscope using Cy5 filter set

**Important**

Thaw at room temperature before starting the experiment.

**KEY PARAMETERS**
**Flow cytometer**

Excitation 633/647 nm laser  
 Emission 660/20 nm filter  
 Instrument specification(s) APC channel

**Fluorescence microscope**

Excitation Cy5 filter set  
 Emission Cy5 filter set  
 Recommended plate Black wall/clear bottom

**PREPARATION OF STOCK SOLUTIONS**

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

**Cell Meter™ RX660 stock solution (500X)**

Add 200 µL of DMSO into the Cell Meter™ RX660 vial to make 500X stock solution.

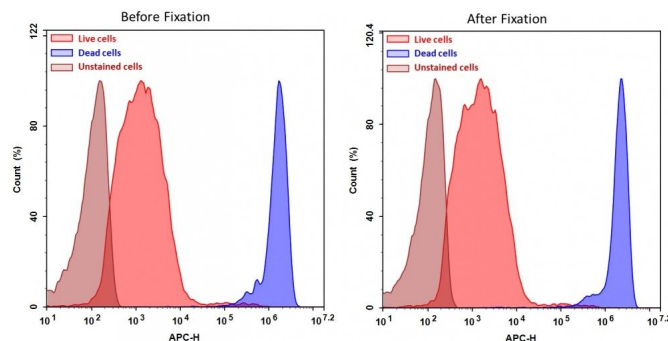
**Note** The unused Cell Meter™ RX660 stock solution should be divided into single use aliquots and stored at -20 °C. Avoid repeated freeze/thaw cycles.

**SAMPLE EXPERIMENTAL PROTOCOL**

1. Treat cells as desired.
2. Wash cells once with HHBS or the azide- and serum/protein-free buffer of your choice.
3. Resuspend cells at  $5 - 10 \times 10^6$  /mL in HHBS or in the azide- and serum/protein-free buffer of your choice.
4. Add 1 µL of 500X Cell Meter™ RX660 stock solution to 0.5 mL of cells/assay and mix it well.
5. Incubate at room temperature, 5% CO<sub>2</sub> incubator for 20 - 60 minutes, protected from light.

**Note** The optimal stain concentrations and incubation time should be experimentally determined for different cell lines.

6. Wash cells twice and resuspend cells with HHBS or the buffer of your choice.
7. Fix cells as desired (optional).
8. Analyze cells with a flow cytometer using 660/20 nm filter (APC channel) and/or fluorescence microscope using Cy5 filter set.

**EXAMPLE DATA ANALYSIS AND FIGURES**


**Figure 1.** Detection of Jurkat cell viability by Cell Meter™ fixable viability dye. Jurkat cells were treated and stained with Cell Meter™ RX660 (Cat#22530), and then fixed in 3.7% formaldehyde and analyzed by flow cytometry. The dead cell population (Blue peak) is easily distinguished from the live cell population (Red peak) with APC channel, and nearly identical results were obtained before and after fixation.

**DISCLAIMER**

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