Cell Meter™ BX650 fixable viability dye

Catalog number: 22520
Unit size: 200 Tests

Component	Storage	Amount
Cell Meter™ BX650 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™ BX650 fixable cell stain is optimized to be excited with the blue laser at 488 nm with emission at 650 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™ BX650 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 695/40 nm filter (PerCP channel) and/or fluorescence microscope using Texas Red filter set

Important
Thaw at room temperature before starting the experiment.

KEY PARAMETERS

Flow cytometer

Excitation	488 nm laser
Emission	695/40 nm filter
Instrument specification(s)	PerCP channel

Fluorescence microscope

Excitation	TexasRed filter set
Emission	TexasRed 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™ BX650 stock solution (500X)
Add 200 µL of DMSO into the Cell Meter™ BX650 vial to make 500X stock solution.

Note The unused Cell Meter™ BX650 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 × 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™ BX650 stock solution to 0.5 mL of cells/assay and mix it well.
5. Incubate at room temperature, 5 % CO_2 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 695/40 nm filter (PerCP channel) and/or fluorescence microscope using Texas Red filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

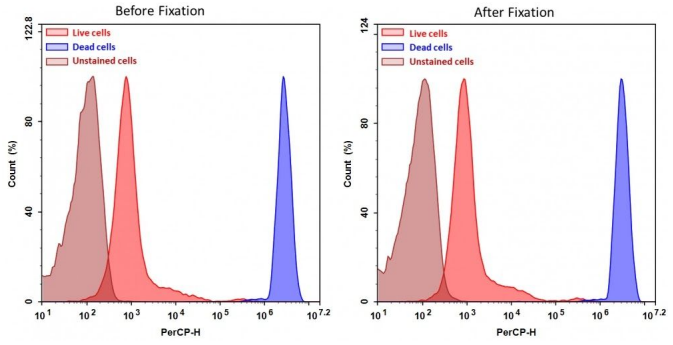

Figure 1. Detection of Jurkat cell viability by Cell Meter™ fixable viability dye. Jurkat cells were treated and stained with Cell Meter™ BX650 (Cat#22520), and then fixed in 3.7% formaldehyde and analyzed by flow cytometry. The dead cell population (Blue peak) is easily distinguished from the live cell population (Red peak) with PerCP channel, and nearly identical results were obtained before and after fixation.

DISCLAIMER

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