Miltenyi Biotec

Viobility™ Fixable Dyes

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1. Description

This product is for research use only.

Products

Dye	Order no. 100 tests	Order no. 500 tests
Viobility 488/520 Fixable Dye	130-109-812	130-110-207

Capacity 100 tests for up to 10^9 total cells

or

500 tests for up to 5×10^9 total cells.

Product format For 100 tests: 1 vial lyophilized dye and 100 µL anhydrous DMSO.

For 500 tests: 5 vials lyophilized dye and 5× 100 µL anhydrous DMSO.

Storage Store dry and protected from light at –20 °C. The expiration date is indicated on the vial labels. For information about reconstitution and storage after reconstitution refer to chapter 2.1.

1.1 Background information

The Viobility Fixable Dyes have been developed for the detection and discrimination of dead cells. They react with the primary amine groups of proteins which can be found on the cell surface as well as intracellularly. In contrast to viable cells, dead cells exhibit a compromised cell membrane allowing the Viobility Fixable Dyes to enter the cell and stain proteins within the cell. This results in a up to 50-fold brighter fluorescent staining and thus allows the discrimination of dead and viable cells by using a flow cytometer.

1.2 Applications

- Analysis of cell viability and exclusion of dead cells by flow cytometric analysis.
- Labeling of dead cells before fixation.

1.3 Recommended dye dilution

The recommended dye dilution is **1 µL for up to 10^7 cells/100 µL** of phosphate-buffered saline (PBS) for staining of dead cells prior to downstream labeling of cells for flow cytometric analysis.

1.4 Reagent requirements

- Phosphate-buffered saline (PBS) without azide, protein, or other amine-containing compound, pH 7.2.
- PEB buffer: Prepare a solution containing PBS, pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

2. Protocols

2.1. Reconstitution

Let the vials warm up to room temperature to avoid water condensation. Reconstitute one vial of lyophilized Viobility Fixable Dye by adding 100 µL anhydrous DMSO to the dye vial and mix until fully dissolved. Aliquot the solution and store at –20 °C under anhydrous conditions (desiccant) and protected from light for up to 1 month.

2.2 Staining with Viobility Fixable Dye prior to staining surface or intracellular markers

▲ Volumes given below are for **up to 10^7** nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Wash cells in 1 mL of 1× PBS and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cells in 100 µL of 1× PBS.
4. Add 1 µL of Viobility Fixable Dye.
5. Mix well and incubate for 15 minutes in the dark at room temperature.

   ▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.

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6. Wash cells by adding 1 mL PEB buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) Repeat step 6.
8. Proceed with surface or intracellular staining as described in the respective antibody data sheet.

2.4 Staining with Viobility Fixable Dye and staining surface markers simultaneously

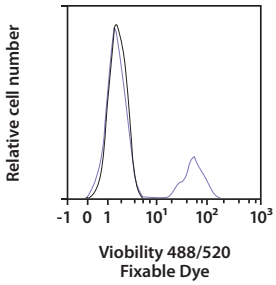
▲ Volumes given below are for **up to 10^7** nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Add 1 µL of Viobility Fixable Dye to 100 µL 1× PBS.
2. Determine cell number.
3. Wash cells in 1 mL of 1× PBS and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
4. Resuspend cells in the prepared solution from step 1.
   The final volume after addition of antibodies (step 6) should be 110 µL, for example, when using 30 µL of antibodies, use 80 µL solution.
5. Mix well and incubate for 5–10 minutes in the dark at room temperature.

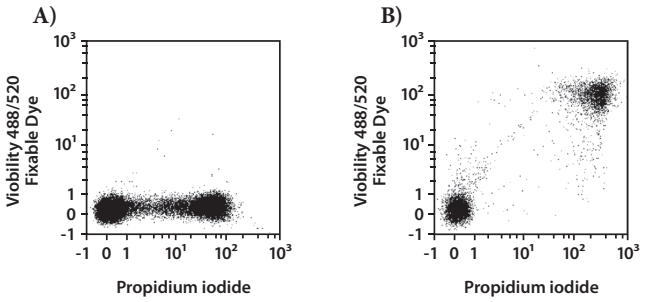
   ▲ Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Add antibodies and incubate for 10 minutes in the dark at room temperature.
7. Wash cells by adding 1 mL PEB buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. (Optional) Repeat step 7.
9. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Examples of dead cell staining with Viobility Fixable Dyes

A mixture of live and heat-treated (for 10 minutes at 95 °C) human peripheral blood mononuclear cells (PBMCs), either unstained (black line) or stained with Viobility 488/520 Fixable Dye (purple line), were analyzed by flow cytometry using the MACSQuant® Analyzer.



A mixture of live and heat-treated (for 10 minutes at 95 °C) human PBMCs were stained with propidium iodide (A) or propidium iodide and Viobility 488/520 Fixable Dye (B) and analyzed by flow cytometry using the MACSQuant Analyzer.



Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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