GeneCopoeia™
Expressway to Discovery

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ViaQuant™ Far-Red Fixable Dead Cell Stain Kit
Catalog Number: A023

Table 1. Kit Components and Storage

Material	Amount	Storage	Stability
Far-Red Reactive Dye (Component A)	**4 vials**	• **-20 °C,** • **Desiccate,** • **Protect from light**	**The product is stable for at least 6 months when stored as directed.**
DMSO (Component B)	**250 µL**		

Number of assays: 200 flow cytometry assays.
Approximate fluorescence excitation/emission maxima, in nm: 650/665

Introduction

ViaQuant™ Far-Red Fixable Dead Cell Stain Kit is designed for discrimination between live and dead cells during flow cytometry or microscopy. The assay is based on the reaction of a fluorescent reactive dye with cellular amines. The reactive dye can permeate the compromised membranes of necrotic cells and react with free amines both in the interior and on the cell surface, resulting in intense fluorescent staining. In contrast, only the cell-surface amines of viable cells are available to react with the dye, resulting in relatively dim staining. The discrimination is completely preserved following fixation of the sample by formaldehyde. Moreover, the single-color assay uses only one channel of a flow cytometer, leaving the other channels available for multicolor experiments.

GeneCopoeia offers several ViaQuant™ Fixable Dead Cell Stain Kits (**Table 2**), which are identical except the fluorescent color of the included dye - blue, violet, green, far red. Cells labeled by blue fluorescent reactive dye are excited by UV (350–360 nm), and blue fluorescence is detected at ~450 nm. The violet fluorescent reactive dye requires violet (~405 nm) excitation with fluorescence emission read at ~450 nm. Cells labeled by green fluorescent reactive dye are excited by the 488 nm line of an argon-ion laser; green fluorescence is typically detected in the green channel of the flow cytometer (530/30 nm). The far-red fluorescent reactive dyes are excited at 633/635 nm with fluorescence emission monitored at 665 nm.

Table 2. Spectral Properties of ViaQuant™ Fixable Dead Cell Stains.

Catalog No.	Reactive Dye	Laser Line	Emission Filter	Abs/Em Maxima
A020	Blue reactive dye	355 nm	DAPI	350/450 nm
A021	Violet reactive dye	405 nm	DAPI	405/450 nm
A022	Green reactive dye	488 nm	FITC	495/520 nm
A023	Far-Red reactive dye	633 nm	Cy5	650/665 nm

Materials Required but Not Provided

- Phosphate buffered saline (PBS)
- PBS with 1% bovine serum albumin (BSA)
- 4% Formaldehyde in PBS
- 0.1% Triton X-100 in PBS

Experimental Protocols

Dye Reconstitution

Remove one vial of dye and the anhydrous DMSO from the freezer and bring to room temperature. Add 50 μL of anhydrous DMSO to the vial, vortexing or pipetting up and down to ensure that all of the dye has dissolved. Once dissolved, the dye should be used within a few hours. Leftover dye solution can be aliquoted and stored desiccated at -20°C for at least 1 month.

Cell Staining for Live/Dead Discrimination by Flow Cytometry

This staining protocol was optimized using Jurkat lymphocyte cell line. The protocol may need to be optimized for other cell types.

1.1 Grow cells in culture as required for your experiment. For adherent cells, detach from the plate using trypsin or a cell dissociation reagent. Count the cells. It is desirable to use at least 1 x 10^6 cells per staining reaction.

1.2 (**Optional**) If positive control (dead) cells are needed, incubate cells at 56°C for 45 minutes, then allow to cool to room temperature and proceed with the protocol.

1.3 Pellet the desired number of cells by centrifugation at 350 x*g* for five minutes and gently pour off supernatant. For all subsequent steps, pellet cells by centrifugation after each incubation or wash.

1.4 Wash cells once in PBS, and resuspend in PBS at a concentration of 1 x 10^6 cells/mL.

Note: Do not wash or resuspend cells in FACS wash buffer containing BSA or serum at this step, because the protein in the FACS wash buffer could interfere with subsequent reactive dye staining.

1.5 Aliquot cells into FACS tubes, 1 mL (1 x 10^6 cells) per tube.

1.6 Add 1 uL of Fixable Dead Cell Stain to 1 mL cells and immediately mix well.

1.7 Incubate for 30 minutes at room temperature or on ice, protected from light.

1.8 Wash cells once with 1 mL PBS.

Note: To stain for surface antigens, proceed to step 1.9. For fixation and intracellular staining, skip to step 1.10. Otherwise, skip to step 1.13.

1.9 Stain for surface antigens:

a. Add the appropriate primary antibodies to cells in PBS.
b. Incubate for 15 minutes on ice or at room temperature in the dark.
c. Wash cells twice with 1 mL PBS.
d. If necessary, repeat steps a-c with the appropriate secondary antibodies.
e. Proceed to step 1.10 for fixation, otherwise, skip to step 1.13.

1.10 Fix cells in 4% formaldehyde for 20 minutes at room temperature.

1.11 Wash cells twice with 1 mL PBS with 1% bovine serum albumin. Proceed to step 1.12 for intracellular staining, otherwise, skip to step 1.13.

1.12 Perform intracellular staining:

a. Resuspend cells in 100 μL PBS + 0.1% Triton X-100.
b. Add the appropriate primary antibodies to cells in permeabilization buffer.
c. Incubate for 30 minutes at room temperature in the dark.
d. Wash twice with 1 mL PBS with 1% bovine serum albumin.
e. If necessary, add the appropriate secondary antibodies to cells in wash buffer and repeat steps c-d.

1.13 Resuspend cells in 1 mL PBS with 1% bovine serum albumin and analyze by flow cytometry in the appropriate channels (see Table 2).

Note: Stained and fixed cells may be stored at 4°C in the dark for several days prior to analysis.

Protocol for Live/Dead Discrimination by Microscopy

This staining protocol was optimized using the adherent human HeLa cell line. The protocol may need to be optimized for other cell types.

2.1 Grow cells in culture as required for your experiment. For adherent cells, staining can be done in a chamber slide, in a multiwell plate, or on a cover slip.

2.2 (**Optional**) If a positive control well containing a mixture of live and dead cells is desired, to that well add ethanol to a final concentration of 15%, incubate for 10 minutes, and wash once with PBS. Replace with PBS or growth media and proceed with the protocol.

2.3 Wash cells with PBS and replace media with PBS containing 1X Fixable Dead Cell Stain.

2.4 Incubate cells for 30 minutes at room temperature or on ice, protected from light.

2.5 Wash cells once with PBS.

Note: To fix and permeabilize cells for immunofluorescence, proceed to step 2.6. For live cell imaging, skip to step 2.11.

2.6 Fix cells in 4% paraformaldehyde for 15 minutes at room temperature, protected from light.

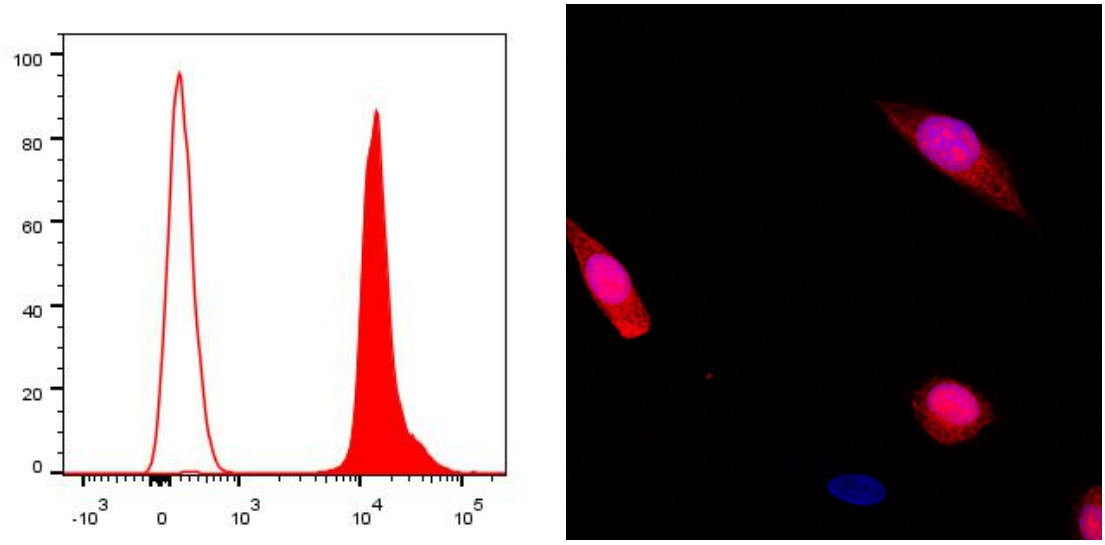
2.7 Wash cells twice with PBS.

2.8 Permeabilize with PBS + 0.1% Triton X-100 for 10 min at room temperature, protected from light.

2.9 Proceed with the immunostaining of your choice. Cells can also be stained with an appropriate DNA dye such as DAPI or Hoechst.

2.10 Wash cells once more in PBS.

2.11 Cells can be imaged immediately on the chamber slide or dish, or alternatively can be mounted using an antifade mounting medium.



Far-Red Fixable Dead Cell Stain

Figure 1. Discrimination of live and dead cells by flow cytometry and fluorescence microscopy using Far-Red Fixable Dead Cell Stains. Jurkat cells were left untreated or killed by heating to 56°C for 45 minutes, then stained according to the product protocol. Fluorescence was analyzed on a BD LSRII flow cytometer (left) and Zeiss fluorescence microscope.